Draft
Generic Protocol for the Verification of Ballast Water Treatment Technologies

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FOREWORD

In 1995, the U.S. Environmental Protection Agency (EPA) instituted a program, the Environmental Technology Verification (ETV) Program, to verify the performance characteristics of commercial-ready environmental technologies through the evaluation of objective and quality-assured data. Managed by EPA's Office of Research and Development, ETV was created to substantially accelerate the entrance of innovative environmental technologies into the domestic and international marketplaces. The independent technology verifications generated through the ETV Program provide purchasers and permitters of technologies with an independent and credible assessment of the technology they are purchasing or permitting.

During its pilot phase, EPA cooperatively managed twelve ETV pilots in conjunction with partner organizations, including states, federal laboratories, associations, and private sector testing and standards organizations. The pilots focused on major environmental media and various categories of environmental technologies. Upon completion of the pilot phase, the ETV Program established six centers, which will carry on with the efforts initiated in the pilot phase. As with the pilots, each center is guided by the expertise of at least one Stakeholder Advisory Group. Stakeholder Advisory Groups consist of representatives of all verification customer groups for a particular technology sector, including buyers and users of the technology, developers and vendors, state and federal regulatory personnel, and consulting engineers. All technology verification activities are based on testing and quality assurance protocols developed with input from the major stakeholder/customer groups. The goal of the ETV Program is that each center becomes a self-sustaining operation by continuing verification activities started in the pilot phase.

NSF International is an independent, not-for-profit organization, dedicated to public health, safety, and protection of the environment. NSF develops standards, provides educational services, and offers superior third-party conformity assessment services, while representing the interests of all stakeholders. In addition to well-established standards-development and certification programs, NSF specifically responds to and manages research projects, one-time evaluations and special studies.

NSF is the verification partner organization for two centers under EPA's ETV Program: Drinking Water Systems and Water Quality Protection. This Protocol for the Verification of Ballast Water Treatment Technologies was developed under the Water Quality Protection Center, whose goal is to verify the performance of technologies used to protect ground and surface waters from contamination. Testing conducted under the ETV program using this protocol does not constitute an NSF or EPA certification of the product tested. Rather, it recognizes that the performance of the equipment has been determined and verified by these organizations.

Verification differs from certification in that it employs a broad, public distribution of test reports and does not use pass/fail criteria. In addition, there are differences in policy issues relative to certification versus verification. Certification, unlike verification, requires auditing of manufacturing facilities, periodic retesting, and mandatory review of product changes and use of

the NSF Mark. Both processes are similar, however, in regard to having standardized test methods and independent performance evaluations and test result preparation. This protocol is subject to revision; please contact NSF to confirm this revision is current.

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Draft Final Generic Protocol for the Verification of Ballast Water Treatment Technologies

FIGURES

TABLES

Glossary of Terms

Accuracy: The degree of agreement between an observed value and an accepted reference value, including a combination of random error (precision) and systematic error (bias) components that are due to sampling and analytical operations (EPA, 1992).

Bias: The systematic or persistent distortion of a measurement process that causes errors in one direction.

Comparability: The measure of the confidence with which one data set can be compared to another.

Completeness: The amount of data collected as compared to the amount needed to ensure that the uncertainty or error is within acceptable limits.

Core Parameters: The measurements that are required as part of the ETV verification.

Effluent: The treated product liquid stream produced by a ballast water treatment technology.

Enrichment Technique: Viability testing incorporating optimal growth conditions, using a series of dilutions.

Equipment: Testing equipment for use in the Verification Testing Program, defined as either a package or a modular system.

Indigenous Populations: The biological organisms, including bacteria, protests and zooplankton, that are naturally occurring at the Test Facility location.

In-Line Treatment: A treatment system or technology used to treat ballast water during normal flow of ballast uplift or discharge.

In-Tank Treatment: A treatment system or technology used to treat ballast water during the time that it resides in the ballast tanks.

Normally distributed data: Data that meets the following criteria: the data forms a bell shaped curve when plotted as a graph, the mean is at the center of the distribution on the graph and the curve is symmetrical about the mean, the mean equals the median, and the data is clustered around the middle of the curve with very few values more than three standard deviations away from the mean on either side.

Owner: The owner of a test site used for verification testing of a ballast water treatment technology.

Performance Data: Removal efficacy and effluent concentration data for core and supplemental parameters.

Precision: The degree to which a set of observations or measurements of the same property, obtained under similar conditions, conform to themselves. Precision is usually expressed as standard deviation, variance, or range, in either absolute or relative terms (NELAC, 1998).

Protocol: A written document that clearly states the objectives, goals, scope, and procedures for the study. A protocol shall be used for reference during Vendor participation in the verification testing program.

Proxy Measurement: A parameter used in lieu of another measurement (chlorophyll to as a measure of phytoplankton).

Quality Assurance Project Plan (QAPP): A written document that describes the implementation of quality assurance and quality control activities during the life cycle of the project.

Representativeness: The degree to which data accurately and precisely represent a characteristic of a population.

Sensitivity: The capability of a test method or instrument to discriminate between measurement responses representing different levels (e.g., concentrations) of a variable of interest.

Stakeholder Advisory Group (SAG): A group overseen by a Verification Organization consisting of representatives from verification customer groups, technology developers and vendors, the consulting engineer sector, the finance and export communities, and government permitters and regulators.

Standard Operating Procedure (SOP): A written document containing specific instructions and protocols to ensure that quality assurance requirements are maintained.

Start-Up: The period between the time the ballast water treatment technology is activated and when stable operating conditions are achieved.

Stable Operation: The time interval following a start-up period that the ballast water treatment technology performs consistently within the range of Vendor-specified operating conditions.

Surrogate Populations: Biological organisms of known types and abundance added to the challenge water during testing of ballast water treatment technologies.

Supplemental Parameters: A measurement taken that is specific to a particular treatment and augments the results of the core parameter measurements.

Technology Panel: A group comprised of a subset of stakeholders and other individuals with technical expertise in ballast water issues, such as scientists, engineers, and ship architects.

Test Plan: A written document that describes the procedures for conducting a test or study according to the verification protocol requirements for the application of a ballast water treatment technology at a particular site. At a minimum, the Test Plan shall include detailed instructions for sample and data collection, sample handling and preservation, precision,

accuracy, goals, and quality assurance and quality control requirements relevant to the particular site.

Test Cycle: One ballasting cycle (including appropriate holding periods) designed to gather data on treatment efficiency.

Testing Organization: An organization qualified to conduct studies and testing of ballast water treatment technologies in accordance with protocols and Test Plans.

Upset:

Viability:

Vendor: A business that manufactures, assembles, or sells ballast water treatment technologies.

Verification: To establish evidence on the performance of a ballast water treatment technology under specific conditions, following a predetermined study protocol(s) and Test Plan(s).

Verification Organization: The party responsible for overseeing Test Plan development, overseeing testing activities in conjunction with the Testing Organization, and overseeing the development and approval of the Verification Report and Verification Statement for the ballast water treatment technology.

Verification Report: A written document, typically prepared by the Testing Organization, containing all raw and analyzed data, all quality assurance and quality control (QA/QC) data sheets, descriptions of all collected data, a detailed description of all procedures and methods used in the verification testing, and all QA/AC results.

Verification Statement: A written document, approved by the U.S. Environmental Protection Agency (USEPA), prepared for a verification test conducted under the Environmental Technology Verification (ETV) Source Water Protection Pilot and summarizing the content of the Verification Report.

Verification Test: A complete test of a treatment technology, which includes enumeration of indigenous and surrogate populations in the challenge water and other defined locations in order to determine the efficacy of the technology.

Viability: The ability of an organism to live and reproduce.

Abbreviations and Acronyms

ATP adenosine triphosphate

CT concentration X time relationship (curve) where combinations of concentration and time that achieves desired treatment effect.

m^3 cubic meters

DOC dissolved organic carbon

DOM dissolved organic matter

EPA U.S. Environmental Protection Agency

ETV Environmental Technology Verification

mgd million gallons per day

mg/L milligrams per liter

MSDS material safety data sheets

NSF NSF International (formerly National Sanitation Foundation)

NTU nephelometric turbidity unit

O&M operations and maintenance

OSHA Occupational Safety and Health Administration

ppt parts per thousand

PSU practical salinity units

QA quality assurance

QAPP quality assurance project plan

QC quality control

QMP quality management plan

SOP standard operating procedure

TSS total suspended solids

Chapter 1
Introduction

1.1 The ETV Program

The U.S. Environmental Protection Agency (EPA) established the Environmental Technology Verification (ETV) Program in 1995. The goal of the ETV Program is to promote environmental protection by accelerating the development and commercialization of improved and more cost-efficient environmental technologies through third-party verification, performance reporting, and information dissemination. The ETV Program does not certify or endorse environmental technologies, but rather provides objective, high quality, peer reviewed performance data that can be utilized by customer groups and regulators when selecting or permitting use of an environmental technology. The ETV program consists of six Centers, focusing on multiple areas of environmental concern. The Water Quality Protection Center (WQPC) develops protocols to verify technologies that protect ground and surface water quality by preventing or reducing contamination. Five Stakeholder Advisory Groups (SAGs) have been established to guide WQPC activities in decentralized wastewater treatment, infrastructure rehabilitation, wet weather flow, watershed protection, and ballast water treatment.

Through a formal Memorandum of Agreement (MOA), the U.S. Coast Guard (USCG) and EPA formed a partnership between the USCG and the ETV Program to better facilitate the development of protocols for evaluating the capabilities of ballast water treatment systems, and to provide a pathway to begin the development of technical procedures for approving ballast water treatment systems for installation on ships (Fact Sheet dated June 11, 2001 Ballast Water Agreement with the U.S. Coast Guard).

1.2 Objectives of Verification Testing

The objective of ETV ballast water treatment verification testing is to ***verify the performance characteristics of commercial-ready treatment technologies with regard to specific verification factors, including biological treatment performance, predictability/reliability, cost, environmental acceptability, and safety.*** Given the wide variety of ship and ballast tank types, treatment technologies and treatment configurations, this protocol addresses the use of a land-based testing facility, rather than shipboard testing, to provide comparable conditions for verifying treatment performance. To ensure that consumers and other stakeholders can make informed choices in selecting a treatment option, land-based ETV ballast water treatment technology verification testing will be conducted in a manner that provides information that is comparable to the maximum practical extent.

1.3 Purpose and Scope of the Protocol

The parties involved with ETV testing, including vendors, testing organizations, testing site owners, and verification organizations, can use the information provided in this Protocol as guidance for ballast water treatment technology verification testing. This Protocol provides guidance on the following necessary elements of verification testing:

- Acceptability for the program
- Vendor provided specification and information
- Test Plan development and content

This protocol is intended for verification testing of entire ballast water treatment systems, not individual component technologies that could be combined to form a system. The systems addressed by the protocol could be in many configurations, such as treatment on uplift or discharge, treatment in-transit (in-tank), or combinations of these options.

Periodic review and revision of Protocols is a critical aspect of the ETV Program. This Protocol will be reviewed and revised, as necessary, following the initial round of testing, and periodically following the first review, to keep the Protocol scientifically and functionally up to date.

1.4 Verification Testing Process

Verification testing is a three-step process, consisting of planning, verification, and data assessment/reporting phases. The planning phase includes development of standardized challenge conditions and the specific experimental design for the test. A site- and technology-specific Test Plan and Quality Assurance Project Plan (QAPP) are prepared during the planning phase in accordance with the guidance provided in Section 4.0 of this protocol. The technology Vendor, Testing Organization, and Verification Organization collaborate on the planning phase documents. The verification phase involves the testing of the treatment technology by the Testing Organization under the conditions and standard operating procedures specified in the Test Plan. In the data assessment and reporting phase, data are processed and analyzed by the Testing Organization, who prepares the draft Verification Report and Verification Statement. The Verification Organization is responsible for QA review of the data generated during the testing, and coordination of the finalization of the Verification Report and Statement.

1.5 Policies and Program Specifications and Guidelines

Treatment technology verification testing will be conducted in accordance the Test Plan, and with the policies, specifications, and guidelines set forth by the ETV Quality Management Plan (for test-specific activities) and the ETV Source Water Protection Pilot Quality Management Plan (for testing activities (http://www.epa.gov/etv/11/11_main.htm).

Chapter 2
Responsibilities of Involved Organizations

Verification testing will typically involve several organizations with responsibilities divided among them. These parties may include the Vendor of the technology, the Testing Organization, the Test Facility Owner, the Verification Organization, EPA, the Technology Panel, and the Stakeholder Advisory Group.

2.1 Vendor

The Vendor of the ballast water treatment technology will apply to ETV for verification testing. The Vendor must provide the Verification and Testing Organization with verification testing objectives and any existing relevant performance data, along with the information required in Section 4.1. This information will be considered during the development of the Test Plan, which will be reviewed and approved by the Vendor. The Vendor will provide a complete system, along with any relevant operations and maintenance manuals, and will be responsible for assuring proper installation and set up of the equipment at the test site. The Vendor will be available for logistical and technical support as required during the planning and verification phases. The Vendor will also be responsible for reviewing the Verification Report and Statement generated from the testing.

2.2 Testing Organization

The Testing Organization is responsible for preparing the Test Plan and working with the Vendor and Verification Organization to gain EPA approval of the Plan, conducting the verification testing and all aspects of test data management, and may be responsible for preparation of the final Report and Verification Statement. The Testing Organization is also responsible for coordinating all personnel and testing activities, operating the Vendor's equipment as specified in the equipment operations and maintenance manual/s, and evaluating and reporting on the performance of the equipment. Maintaining security for testing activities and site safety for all personnel is the responsibility of the Testing Organization.

2.3 Test Facility Owner

If different from the Testing Organization, the Owner of the verification testing facility may provide logistical and technical support during planning and verification phases, as agreed upon by the Testing Organization, Vendor, and Owner. The Owner must notify the Testing Organization of any logistical or operational developments that may affect the verification testing process and results.

2.4 Verification Organization

The Verification Organization is responsible for overseeing the development and approval of the Test Plan, which details study objectives, specific test procedures, and assurance requirements. In addition, the Verification Organization will collaborate with the Testing Organization to administer testing activities at the Test Facility. The Verification Organization is also

responsible for review and gaining EPA approval for the Verification Report, which will contain all raw and analyzed data, data descriptions, details of procedures and methods, and all QA/QC data sheets and results, and the Verification Statement, summarizing the content of the Verification Report. The Report and Statement are typically drafted by the Testing Organization, but may be drafted by the Verification Organization or a contractor to the Verification Organization. The Verification Organization is also responsible for initiating and coordinating periodic review and revision of this Protocol.

2.5 U.S. Environmental Protection Agency

The EPA Office of Research and Development, through the National Risk Management Research Laboratory in Cincinnati, Ohio and the Urban Watershed Management Branch in Edison, New Jersey, oversees the ETV Program. The EPA Center Manager for the Water Quality Protection Center will be responsible for review and approval of Test Plans for ballast water treatment technology verification testing, the Verification Report and Statement generated from the testing, and for assuring that the Verification Report and Statement is posted on the EPA/ETV web site. EPA is also responsible for coordinating review and approval of revisions that may be proposed to this Protocol.

2.6 Stakeholder Advisory Group

Stakeholder Advisory Groups (SAGs) are established in each of the ETV Program's six Centers. SAGs consist of representatives from verification customer groups, such as buyers and users of technology, developers and vendors, the consulting engineering sector, the finance and export communities, and government regulators. The SAGs support generic verification protocol development, prioritizing the types of technologies to be verified, and defining and conducting outreach activities appropriate to the technology area and customer groups. In addition, the SAGs may review WQPC-specific procedures and selected ETV verification reports emerging from the ETV Center and serve as information conduits to the particular constituencies that each member represents. The Ballast Water SAG, of the Water Quality Protection Center, is charged with addressing ballast water treatment technologies.

2.7 Technology Panel

The Technology Panel is comprised of a subset of stakeholders and other individuals with technical expertise in ballast water issues. Scientists, engineers, technology vendors, naval architects and regulators supported the development of this Verification Testing Protocol. In the future, the Technology Panel may be responsible for reviewing the technology specific Test Plans, and Verification Reports and Statements. The Panel will also be responsible for working with the Verification Organization in reviewing and revising this Protocol, as needed.

Chapter 3
Ballast Water Treatment Technology Capabilities and Description

3.1 Ballast Water Treatment Technology Definition

For the purposes of this verification testing program, ballast water treatment technologies are defined as:

> Prefabricated, commercial-ready, treatment systems designed to remove, kill or inactivate organisms that are potentially harmful to human health and receiving ecosystems from ballast water prior to discharge.

This definition includes both in-line (systems that treat the flow of ballast either on uplift or discharge) and in-tank systems (systems that treat ballast water during the time that it resides in the ballast tanks). Typically, ballast water treatment systems treat an average design flow between 1.4 – 17 m^3 per minute (350 – 4,400 gpm) or a total tank volume within a range of 20 – 14,500 m^3 (5,280 – 3,828,000 gals.).

Treatment technologies that will be tested under this program will be capable of treating the entire discharge or ballast water volume for biological organisms, either through a one step treatment process or through a multi-step treatment process, and will be capable of treating a wide range of source water typical to ballast uplifted from fresh, coastal, estuarine and marine origins. These technologies may be biological, physical, or chemical in nature or a combination of any or all of the technologies. Treatment systems, or components of systems, that can provide only partial treatment of the discharge are excluded from verification testing.

3.2 Technology or Treatment Performance Objectives

The vendor will supply a statement of treatment performance objectives for the treatment or technology. This will include:

- Quantitative measures of biological treatment performance (direct count, removal efficiency, or measured indicator) for a range of biological functional groups including microorganisms (bacteria, and viruses), and macroorganisms (including holoplankton meroplanktonic, demersal, and pelagic organisms and life stages). Minimum reporting parameters are specifically detailed in Section 5.7.
- The required operational and maintenance conditions (operator time, power requirements, chemical requirements, reliability, etc.) to achieve the biological performance under a range of source water conditions typical to fresh, coastal, estuarine, and marine ballast water (water conditions are detailed in Section 5.3.1).

3.3 Acceptability for Testing

The treatment technology must meet the definition of a ballast water treatment technology, meet all existing environmental regulatory requirements for operation and treatment byproduct discharge, and must be safe to operate for the crew and vessel. Only complete treatment systems

will be accepted for verification testing. Treatment components designed to provide only pretreatment or primary, secondary or tertiary treatment will not be accepted for verification testing.

The Verification Organization has the right to reject a proposed treatment system that does not satisfy the definition of a ballast water treatment system in Section 3.1. A proposed treatment system may also be rejected from acceptance to the verification testing program if, for technical or logistical reasons, it cannot be accommodated in the evaluation.

Chapter 4
Treatment Verification Test Plan Development

4.1 Guidance on the Vendor Provided Description of Environmental Technology

The Vendor must provide a detailed description of the equipment/treatment technology for inclusion in the Test Plan, including:

- Engineering description (e.g., construction and components, power requirements, flow or volume capacity, dimensions, weight, flange sizes, required connections, specificity to particular ballasting configurations)
- Process description including performance ranges and expectations
- Discharge characteristics
- Footprint
- Photographs
- Process/technology limitations
- Costs to install, maintain, and treat
- All relevant data from prior tests

Vendor must also include a detailed operation, maintenance, and safety manual with the approved Test Plan that includes:

- Start-up procedures and time
- Length of operation to achieve treatment objectives
- Operation and maintenance instructions, cycle time, and materials (expendables)
- Labor requirements (time, level, and training)
- Safety issues, hazards, and warnings (i.e., OSHA certification as applicable and other safety certification as appropriate, MSDS, etc.)
- Any special requirements for use
- Safety emergency instructions
- Environmental hazards
- Waste disposal procedures
- Troubleshooting guide
- Point of contact name and phone number/email for technical questions
- Recommended spare parts to have prior to testing

4.2 Required Elements of the Test Plan

Each ballast water treatment verification test will be completed following a written Test Plan. The Test Plan will detail study objectives, specific test procedures (including sample and data collection, sample handling and preservation) and quality control and assurance requirements (including measures of precision, accuracy, comparability, and representativeness). The experimental approach for the ballast water treatment test, treatment system start-up, and verification procedures will be presented in the Test Plan. The Test Plan will include a summary description of the standardized water quality and biological challenge conditions established by the experimental design protocol included in Section 5.3. The Plan will summarize how the

challenge conditions will be implemented at the testing facility relative to the ballast water treatment technology being tested. Any modifications or supplements to the treatment verification protocols will be defined and discussed in the Plan. The Test Plan will also address quality assurance/quality control (QA/QC) requirements, data handling and presentation, and environmental, health, and safety issues.

The Testing Organization is responsible for preparing the Test Plan, with input from the Vendor. The Verification Organization shall review and coordinate the approval of the Test Plan prior to the start of verification testing.

The Test Plan shall include:

- Title page/approval page with all project participants
- Table of contents
- Project description and treatment performance objectives
- Project organization and personnel responsibilities
- Test facility description
- Treatment technology description
- Experimental design (including installation/start-up plan)
- Challenge water conditions and preparation (including test facility SOPs for preparation)
- Sampling and analysis plan including sampling and analytical procedures
- Quality assurance project plan (QAPP)
- Data management, analysis and reporting
- Environmental, health and safety plan
- References
- Appendices (including vendor operation and maintenance manual)

Content requirements for the Test Plan are discussed in more detail in Appendix A.

Chapter 5
Experimental Design

The purpose of ETV ballast water treatment verification program is to verify the environmental performance characteristics of ballast water treatment technologies. Other factors pertinent to the treatment technology's performance will also be evaluated. To ensure that consumers and other stakeholders can make informed choices in selecting a treatment option, land-based verification testing will be conducted in a manner that provides comparable information to the maximum practical extent. One way to ensure this is to use a standard set of challenge conditions when testing each treatment technology. Standardized challenge conditions included in this protocol address both water quality conditions and the biological organisms used to evaluate treatment performance. Key water quality challenge conditions are standardized under this protocol because the effectiveness of many treatments may be influenced by certain water quality characteristics (e.g., salt, turbidity, color, etc.). Moreover, the natural environment has a large range of conditions, which may or may not provide adequate information on a treatment's ability to perform adequately under non-ideal water quality conditions. Therefore, non-ideal water quality conditions form the basis for challenging the treatment systems. Towards this end, the protocol also includes the analysis of indigenous species and a set of surrogate biological species to measure treatment efficiency. Surrogate species are included to better evaluate the effectiveness of treatments on organism life stages known to be resilient under test conditions and to provide a means of comparing among technologies and tests conducted at different locations and dates.

The following sections provide guidance on the five key elements of the protocol: 1) test verification factors, 2) water quality and biological challenge conditions, 3) flow rates and volumes, 4) start-up requirements, and 5) verification testing, including the measurement program required under this protocol. Variations in the protocol for specific treatment technology types (e.g., in-line treatment versus in-tank treatments) are also described.

5.1 Test Verification Factors

All treatment systems will be verified according to the following factors:

- Biological treatment performance
- Operation and maintenance
- Reliability
- Cost factors
- Environmental acceptability
- Safety

Biological treatment performance - defined as the removal, inactivation, or death of organisms. Performance can be measured in terms of removal efficiency (e.g., a percentage) or against a threshold (e.g., a water quality standard). The measurement program required by the protocol evaluates this by measuring the viability of organisms passing through the treatment (potential to survive and reproduce after treatment (regrowth)).

Operation and maintenance - includes the labor, expertise required to operate, equipment and consumables required to operate the system to achieve the stated performance goals and objectives.

Reliability - a statistical measure of the number of failures (either qualitative or quantitative) per known quantity of test cycles.

Cost factors - include only those factors that can be verified such as operating costs.

Environmental acceptability - assesses ballast water quality following treatment for factors other than the abundance and viability of organisms. For example, this will determine if the treated water meet acceptable water quality characteristics for such measures as dissolved oxygen, temperature, treatment residuals, pH, etc. Environmental acceptability ensures that treated water does not adversely affect the environment when discharged. At a minimum, the verification test will ensure that the discharge of treatment residuals meets federal and state regulations for wastewater discharge.

Safety factors - includes any treatment specific considerations that may pose a threat to the safety of the operator or shipboard operations.

Performance test results will be reported using standard ETV formats to make certain the reported information among treatment technologies tested is comparable. Flexibility is permissible to ensure reporting for a specific treatment technology type is appropriate and accurate.

Some information supplied by vendors may not be verified under the protocol. However, such information can be included in the Verification Test Report as non-verified information. This may include such information as shipboard compatibility (e.g. corrosion resistance, system weight, system volume [includes clearances needed to perform maintenance and replace vital components], compatibility with other common shipboard systems such as operational flow rates). Submission and reporting requirements for non-verified, vendor-supplied information is included under Sections 4.1 and 4.2.

5.2 Objectives of Verification Testing

The general objectives of the verification testing are to:

- Provide a comprehensive set of water quality and biological challenge conditions against which treatment effectiveness can be quantitatively evaluated.
- Develop adequate data to document system performance against the verification factors.

The requirements for testing are described in the following sections. Section 5.5 provides guidance on the biological treatment performance testing.

5.3 Challenge Conditions

This protocol recognizes that land based testing cannot fully replicate actual treatment technology performance onboard ship. However, land based verification testing can provide sufficient information to verify the expected performance of treatment in the shipboard environment. It is understood that all treatment technologies will face a range of physical/chemical water quality conditions and biological organisms when operated onboard a ship. Therefore, each treatment technology's performance will be verified using a set of standard challenge conditions. Two factors must be addressed to properly challenge treatment technologies: water quality and biological organisms. This protocol defines the following objectives for the challenge conditions:

- To verify a treatment system's performance using a set of extreme, but not rare, water quality conditions representative of the natural environment.
- To verify removal, kill, or inactivation of bacterial, protists, and zooplankton species using indigenous and surrogate species, and analytical techniques that test survival and growth.

5.3.1 Challenge Water – Water Quality Characteristics

Since water quality conditions in ports and harbors around the world vary greatly, treatment systems may encounter a wide range of water quality conditions. Also, certain water quality conditions are known to interfere with the ability of some treatment processes. It is therefore critical to evaluate the effectiveness of a treatment system under water quality conditions that are difficult to treat. Simulating all potential water quality conditions in a land-based testing design would be prohibitively expensive[1] and not essential for verifying the performance of a treatment system. Because water quality conditions that can interfere with various treatments are generally understood and few in number (e.g., salinity, turbidity, organic matter either as dissolved or particulate forms), the number of water quality parameters that must be explicitly included in the protocol can be limited. Thus, this protocol defines two challenge conditions that represent some of the more extreme, natural conditions that may be encountered by ballast water treatment systems. Challenge water quality characteristics to be used during testing events are presented in Table 5-1.

Another basic premise in the design for this protocol is that ballast water treatment systems are designed to handle the full range of water quality characteristics that will be encountered under operational conditions. By challenging the treatment systems with extreme conditions it is assumed treatment will be effective under less extreme conditions. Challenge waters have been tailored to include natural water conditions[2] and a set of simulated conditions[3] that provide an extreme challenge to treatment systems. The challenge conditions are composed of two levels of salinity, <1 and 28 to 33 PSU (Practical Salinity Units), and water quality characteristics that are

[1] Similarly, shipboard testing of all potential water quality conditions will require extensive logistics to move a treatment system to a matrix of natural conditions, as well as investment in methods and protocols by which the treatment effectiveness is established using natural populations of organisms.
[2] Natural conditions are evaluated over the duration of the Verification Test.
[3] These conditions will be implemented during the surrogate additions described in Section 5.3.2.

commonly cited as a problematic for the range of technologies being developed to treat ballast water: 1) solids, 2) dissolved organic matter[4].

Table 5-1. Water Quality Challenge Matrix for Verification Testing

Water Type	Water Quality Characteristics
Fresh (Salinity <1 PSU)	**DOM/DOC:** 8-12 mg/L as DOC **POM:** 8-12 mg/L **MM:** 16-22 mg/L **Sum of POM + MM:** 24-34 mg/L **Temperature:** 10 – 35 °C
Marine (Salinity 28-33 PSU)	**DOM/DOC:** 8-12 mg/L as DOC **POM:** 8-12 mg/L **MM:** 16-22 mg/L **Sum of POM + MM:** 24-34 mg/L **Temperature:** 10 – 35 °C

In nature, solid material that interferes with treatment effectiveness is composed of several types of particles. These include particles of biological origin and those that are of mineral origin, specifically clay and silt. The water quality challenge condition defined by the solids content of the matrix includes particulate organic matter (POM) and mineral matter (MM). These two forms of particles are both present in natural waters at a range of concentrations. Both forms are included in the challenge conditions to address issues of particulate removal and turbidity, which can interfere with transmission of light or other treatment actions.

Various forms of dissolved chemicals and compounds, particularly organic material can directly affect the efficiency of some treatment processes. Dissolved organic matter (DOM) and dissolved organic carbon (DOC) are two terms used to describe this component of natural water. DOM/DOC often contains many chromophors that contribute substantially to the color of the water, another potential interference for treatments. Thus, the color of a water and DOM/DOC concentration are often interrelated.

The measurement methods for evaluating the status of the challenge conditions are described in Section 5.7. The measurements include standard analytical methods to document the concentration of total suspended solids, particulate organic matter or dissolved organic matter, and methods that indirectly measure these parameters (e.g., turbidity measured by electronic/optical measurement such as nephelometery (NTU's) or transmissometry (beam attenuation) or fluorescence (color /DOM)).

[4] The protocol does not explicitly call for verification at a series of temperatures, even though some treatments may have strong temperature dependence or temperature manipulation may be part of the treatment procedure. Rather than include temperature as a controlled water quality condition, which can have significant cost implications for the Test Facility, accurate and continuous monitoring of the source and treated water temperatures is required for all test cycles. If temperature manipulations are included, the Test Plan will include protocols for these manipulations.

Standardization of the water quality conditions for the verification testing requires a consistent set of source water (e.g., fresh and marine water), as well as use of well-characterized organic matter and mineral matter. The Test Facility will be responsible for providing these materials and ensuring the water quality conditions are as described under this protocol. The water quality test conditions will be standardized for salinity, particulate organic matter, mineral matter, and dissolved organic matter as follows:

Salinity - Natural water of less than 1 PSU will be used for the fresh water condition, while natural seawater will be used for the high salinity condition. If the salinity is less than 28 PSU, the salinity will be raised using commercially available ocean salt.

Particulate and Dissolved Organic Matter - Organic matter in the form of terrestrial humic matter is commercially available in several forms[5] and will be used as the source of POM. Since this material also dissolves to a degree when added to water, it will contribute to the dissolved form of the organic matter in the challenge water. Particulate carbon from sources such as ground up seaweed or plankton detritus may be included in the testing.

Mineral Matter (MM) - Clays and Silts - Mineral particles in the size range typically found in coastal and estuarine waters are readily obtained from commercial sources and will be used as the source of the mineral matter. Studies of sediment size in ballast tanks suggest that particles are mostly fine grained (less than 60 micron) with very little sand present (F. Dobbs, personal communication, October 2001). Thus, addition of the commercially available clay minerals (with a majority of particles in the 10 to 50 micron size) addresses the objective of having a prescribed level of non-biological particles as part of the water quality challenge condition.

The Test Facility will be responsible for preparing the challenge water and documenting the challenge conditions. Challenge waters will be prepared under Standard Operating Procedures developed by the Test Facility. The Test Plan will include these SOPs and describe any planned deviations from the SOPs.

5.3.2 Challenge Water - Biological Organism Conditions

The inactivation, death, or removal of viable, living organisms is central to the need to treat ballast water. To ensure proper evaluation of a ballast water treatment system's performance, the effects on biological organisms living in the challenge water will be measured for each treatment system tested. The test organisms will include a set of surrogate species added as part of the test.

[5]Available forms range from fulvic acids (more soluble, yellow in color) through humin (less soluble, black in color). Humic acids that are intermediate in characteristics between these materials will be used for the tests. Large volumes are generally available through garden and agriculture industries as soil amendments and will be provided by the Test Facility to ensure consistent properties.

Surrogate species, shown in Table 5-2, will be added to the challenge water. These species are representative of the three major groups of organisms (microbial/pathogenic[6], heterotrophic and autotrophic protists[7], and mero and holo/zooplankton) encountered by treatment systems in an operational environment. Surrogate species selected for the microbial and phytoplankton communities include those with life stages that are resilient (i.e., endospores or resting forms)[8]. Known numbers of the surrogate species, based on a calculation using the Poisson distribution (described further in Section x.x), will be added to the challenge water ahead of the treatment system. These life stages are believed more difficult to treat, thus potentially pose a greater threat for invasion than the vegetative forms. Surrogate species will be obtained from commercial suppliers to the maximum extent possible. If surrogate species not available from commercial sources, specific surrogate cultures may be maintained at the Test Facility. Spores and resting forms provide logistical advantage in that once cultured, they can be kept in storage for several months. The viability[9] of each surrogate shipment will be tested at the Test Facility upon receipt from the vendor. Each surrogate stock will be assayed for the number of live organisms and viability within 24-hours of the start of a test cycle. If the test cycle is to be conducted more than one week after receipt of the organisms, the surrogate batch will be re-assayed. Surrogate vendor methods will be used for these assays. At least two surrogate species per biological group will be added to each of the challenge water types.

Table 5-2. Surrogate Species (*Species to be finalized following screening testing*)

Functional Group	Fresh Water	Marine Water
Bacteria	*Same as Marine Water*	*Geobacillus stearothermophilus* *Clostridium perfringens* *Enterococcus avium*
Zooplankton	Daphnia Cladoceran Rotifers	*Acartia hudsonica* (warm water - resting) *Acartia tonsa* (cold water - resting) Oyster (larvae) Sea Urchin (larvae) *Brachionus calyciflorus* (adult) *Tisbe battagliai* (adult)
Protist	*Acanthomeoba* *Fragilaria crotensis*	*Scrippsiella lachrymose* (dinoflagellate) *Acanthomoeba* *Corethron hystrix* *Ditylum brightwelli* *Fragilaria pinnata*

[6] Virus surrogates are not included at this time. Smaller scale research is recommended to establish relationships between efficacious kill of spore-forming bacteria and inactivation of viruses.

[7] Protists are defined as single celled eukaryotic microbes including colorless, heterotrophic 'protozoa', and green autotrophic 'algae'.

[8] A research program has been designed to evaluate and determine the comparability among surrogate species.

[9] Viability in this context means the number of living cells or organisms per unit volume in this context.

5.3.3 Challenge Water – Flow Rates and Volumes

Treatment tests will evaluate equipment at operational flow rates defined by the Vendor's O&M manual. The Test Facility will be capable of providing flow rates of up to 300 m^3 per hour (1,320 gal per minute) for duration of two hours (available volume per test cycle up to 600 m^3). The Test Facility shall provide sufficient challenge water volume to meet these requirements. The Test Plan will identify the rates that will be tested.

The recommended volume for in-tank testing is also 300 m^3 (~79,000 gallons). The Test Facility shall provide test and control ballast tank configurations of up to 300 m^3. Larger volumes may be used depending on Vendor specifications and availability of tanks at the Test Facility. A range of tank sizes and ballasted configurations (e.g., partially full versus full, etc.) may be verified. The size(s), configuration(s), and rationale for partial tank volume testing will be defined in the Test Plan.

5.4 Start-up

The objectives of the start-up task are to:

- Install and start the ballast water treatment system in accordance with the Vendor O&M manual;
- Reach stable operating conditions;
- Make modifications as needed to ensure stable operations under test facility conditions; and
- Record and document all installation and start-up conditions, observations and results.

The treatment system shall be installed at the Testing Facility according to the Vendor instructions included in the Test Plan. System installation testing will be conducted to ensure successful installation. Once successful installation has been confirmed, the system will be designated as operational and verification testing will begin. The ballast water treatment system will be operated according to the Vendor instructions as provided in the O&M Manual.

5.4.1 Operation and Maintenance (O&M) Manual

The O&M manual shall be incorporated into the Test Plan and will be key to the development of the monitoring and maintenance plan to be incorporated into the Test Plan. The Vendor shall identify factors that affect the operation of the unit, including any warm up or other requirements that must be completed for operational stability to be achieved. The Vendor's O&M manual shall specify what constitutes stable operating conditions for the treatment system, factors that may affect operating conditions, and any adjustments required to reach or to maintain a stable operating condition. Adjustments made in the operating conditions will be presented in the final Verification Testing Report.

5.4.2 Vendor and Test Organization Requirements

An installation/start-up work plan shall be prepared and included as part of the Test Plan. The Testing Organization shall conduct start-up procedures for the ballast water treatment system in accordance this installation/start-up plan and with the Vendor O&M manual. At the end of the start-up period, the Testing Organization will assess whether or not ballast water treatment system is in a stable operating state, as specified in the O&M manual and the Vendor will certify, in writing, that the system is installed and operating as intended. If the operation is stable, the verification testing can begin. If not, start-up procedures will be repeated for up to two more times. If the system does not achieve stable operating conditions after three start-up cycles, the Testing Organization, in conjunction with the Vendor, will review the start-up work plan for applicability and determine where adjustments and modifications are required.

The Vendor will identify any additional equipment, system maintenance, changes to operating conditions, or other modification needed to ensure effective operation of the system, and to attain or maintain stable operational conditions.

Ambient waters shall be used during start-up tests. Challenge water conditions shall only be used during the biological test runs.

5.4.3 Toxicity Testing for Biocide Treatments

The residual toxicity in the discharge from treatments employing a biocide is of concern to the Test Facility. Toxicity of the water following treatment will be addressed as part of the NPDES requirements for the Test Facility. However, to ensure compliance with the facility's NPDES permit requirements, a toxicity evaluation of the treated waters at the end of a verification cycle is warranted, and shall be conducted during the start-up phase of verification testing according to the toxicity methods cited in Section 5.7. If the post treatment effluent passes the toxicity tests, then verification testing can proceed. If, however, the effluent fails the toxicity test, verification testing shall not be initiated and further toxicity tests shall be required. The vendor shall be allowed no more than two additional attempts to pass the toxicity tests within 30 days of the initial test. This may require modifications to the approach for verifying the technology in the Test Plan or other investigations to understand the toxicity response.

5.5 Verification Testing

Ballast water treatment technology performance, operating conditions, and certain O&M criteria will be documented and evaluated during verification testing by the Testing Organization and presented in the Verification Report. The factors to be verified during ballast water treatment system verification testing include: biological treatment performance, operation and maintenance, predictability/reliability, cost factors, environmental acceptability, and safety.

Any of several treatment sequences[10] may be used by a particular treatment system (Table 5-3), including in-line treatment (during ballasting or deballasting), in-tank treatment, or a combination of the two. The stage in the ballasting cycle at which treatment is applied may also vary. This verification testing protocol accounts for these through flexibility in the Testing Facility and Verification Test Plan. The guidance in the following section provides the basic test requirements and rationale for inclusion in the Test Plan that will provide details specific to the treatment system and its operation.

Table 5-3. Likely Treatment Sequences and Applications Inherent to Ballast Operations

Sequence Number	Ballast Operation Application
1	Treatment applied during ballasting/ No treatment during deballasting
2	Treatment applied during ballasting/ Treatment applied during deballasting
3	No treatment applied during ballasting/ Treatment applied during deballasting
4	No treatment applied during ballasting/ Treatment applied during transit/ No treatment during deballasting
5	No treatment applied during ballasting/ Treatment applied during transit/ treatment during deballasting
6	Treatment applied during ballasting/ Treatment applied during transit/ No treatment applied during deballasting

The objectives of the verification testing are to:

- Evaluate the treatment performance of the ballast water treatment system relative to the removal or inactivation of indigenous and surrogate biological species, operating under Vendor-specified conditions;
- Evaluate the treatment technology O&M criteria;
- Determine and record cost factor data, and
- Record and document test conditions, observations, and results.

Other testing objectives may be defined by the Vendor and included in the Test Plan. The requirements for Verification Testing are described in the following sections and must be addressed in the Test Plan.

Because of the potential that verification for both in-tank and in-line treatments may be requested by Vendors, this generic protocol includes two primary approaches for setting verification testing duration. The first is to operate the treatment system for a given number of ballasting cycles as

[10] A simplified ballasting cycle includes three basic stages 1) ballasting (B), 2) transit from port of origin to destination (t), 3) deballasting (D). Treatments may or may not take advantage of the transit time as part of the treatment cycle. Thus, treatment (T) may be applied at one or more of the ballasting steps including while ballasting (T_B); in transit (T_t); and during deballasting (T_D).

defined by the Vendors O&M manual. The second is to conduct the test under a minimum total volume treated. This latter approach may be more appropriate for in-tank treatment. It may also result in testing of less than a full maintenance cycle. The rationale for selecting one or the other of these options must be described in the Test Plan and any implications to the Verification Test's ability to fully evaluate the O&M criteria for the treatment technology must be discussed.

5.6 Verification Test Duration

Testing under the ballasting cycle approach - Each verification test using this approach will be conducted for at least 1½ times (or 150 percent of) the treatment system's designed operation and maintenance cycle, requiring the system to be operated for either a defined number of hours or ballasting cycles. This allows the testing to validate operation of a new unit, as well as after the unit has been operated beyond a maintenance cycle, and provides information about the reliability and predictability of the treatment system. This approach also involves a substantial duration for the testing period and allows testing to be conducted over a range of water temperatures, depending on the location of the Test Facility (e.g., temperate versus a subtropical location). To accommodate the potentially large number of ballasting cycles, biological testing using addition of surrogate species will be required on only six of the test cycles. These full biological test cycles will be conducted at water temperatures between 10 and 35ºC. Water temperature must be reported for each test cycle, but it is not required that tests be conducted at a specific temperature or series of temperatures.

The other ballasting test cycles will provide data on the system's operation and support the assessment of non-biological verification factors. In the case of in-tank treatment approaches, particularly those using biocides or other chemical/physical means of achieving treatment, the Test Plan may elect to test the operation of the equipment at 150 percent of the O&M cycle without including the active agent (i.e., to verify the electro/mechanical aspects of the treatment unit). During actual shipboard operation, ballasting procedures may occur over time periods ranging from minutes to hours. For each in-line treatment verification cycle, a minimum ballasting period of one (1) hour is required. Longer ballasting periods may be required if treatment system flow rates are less than half the standard flow rate of 300 m^3 per hour.

In addition to the ballasting time, holding times will be included in the duration of each full biological test cycle to simulate the time that water would reside in a ballast tank. Thus, the duration of each test cycle will be defined by the operational approach used by the treatment technology. The holding time of the six required full biological test cycles may extend the test by several days. In-tank treatment cycles will also include holding times during each full biological test cycle.

Minimum volume test approach - Under this verification approach, O&M criteria will be evaluated based on the number of ballasting cycles (which may be flow dependent) completed to meet a minimum volume treated requirement. If the duration of testing does not necessitate maintenance of the system, the routine maintenance described in the system's O&M manual shall be completed at the end of the testing. The minimum volume requirement for an in-line verification test is 10,000 m^3. This is equivalent to ~30 hours of operation at 300 m^3 per hr (or ~65 hours of operation at 150 m^3 per hr). For in-tank treatments the minimum volume treated

will be 1,800 m^3, equivalent to six 300 metric ton tests. The volume treated under each test cycle will be defined in the Test Plan. A minimum of six cycles with surrogate species is required.

A schematic diagram of an example ballasting cycle scenario is shown in Figure 5-1. The schematic represents a test period for an in-line verification that includes forty ballasting and deballasting cycles (each assumed to be a 1 hour ballasting operation) with a five-day holding time for each of the six biological test cycles. In the example, the O&M manual calls for major maintenance after 25 hours of operation such that the test duration is 1.5 times 25, or 37 hours. Assuming one test cycle per day with six cycles for surrogate tests, the total test period would cover about 60 working days. Since temperature control is not part of the test protocol, verification testing with surrogate species could be conducted any time when temperatures are between 10 and 35ºC (this ranges represents the temperatures at which most biological activity is not slowed substantially by low temperatures and also recognizes that biological activity doubles for approximately every 10ºC change in temperature). Given the five-day holding time requirement, assuming limited availability of tanks for holding water, and that verification cycles could proceed in parallel with the holding time, the duration of testing could be reduced to approximately 45 days. The timing of the surrogate test cycles must be integrated into the Test Plan.

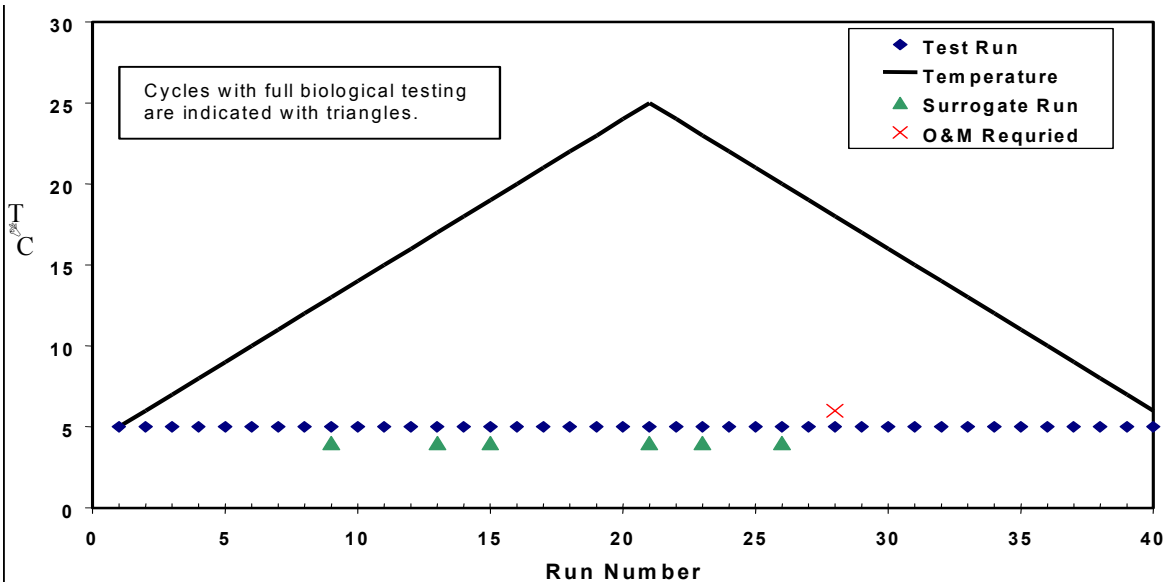

Figure 5-1. Schematic representation of verification test duration.

For in-tank treatments, test duration will include the minimum contact time the Vendor prescribes for effective treatment, plus a five day holding time for each of the six required surrogated test cycles. As with the in-line approach, testing of the equipment without active ingredients may be run in parallel with the surrogate test to reduce the overall duration of the verification test. Modifications may be made according to vendor specified requirements for treatment, but they must be justified in the Test Plan. For example, if holding water for a specified time after the treatment's minimum contact time is required by the Vendor, that time

interval would be added to each verification test incorporating surrogate species. For combinations of in-tank and in-line treatment, test duration will be equal to treatment time (in-line plus in-tank).

The holding time included in this protocol is designed to represent voyage conditions and to address regrowth issues. For in-tank treatment with additional in-line treatment during ballast water discharge, the duration will be equal to in-tank treatment requirements and the deballasting time. Holding times longer than five days may be included in the Test Plan as can testing for viability at increasing time intervals after treatment (during the holding period). Justification for longer holding times must be included in the Test Plan.

5.7 Biological Treatment Performance

Treatment efficiency will be determined from the viability of the surrogate species recovered in the and total abundance of each class of indigenous organisms before and after treatment (at discharge). The significance of treatment efficiency will be evaluated using simple t-tests (Section 9.2). Tests for the viability of the surrogate species will be included in the evaluation of treatment efficiency. Inclusion of organism viability as a testing endpoint will demonstrate that surrogate and indigenous species populations are tested for their ability to grow or reproduce after treatment.

Viability will be tested using enrichment methods (Rogerson and Gwaltney, 2000 and Hall *et al*., 1996) that incorporate multiple media or growth in light or dark conditions, or both, as appropriate to the class of organisms being tested. Enrichment dilution series will be incubated at a constant temperature for 7 to 14 days with a ± scoring of each enrichment sample used to evaluate the response. Standard practices for control and validation of the biological measures will be followed including treatment and analytical controls. Enrichment measures will be established to measure at least a 1 in 100,000 change. Evaluation of indigenous zooplankton species is not required due to variability in natural abundances.

A minimum of six valid verification test cycles (three each of fresh water and marine water) with surrogate species will be conducted for each treatment system submitted for verification testing. A test cycle shall be considered valid if the treatment technology functions per Vendor specifications. The six test cycles will include at least three tests for each of the two challenge conditions defined in Table 5-1. Surrogate species may be injected into the challenge water stream just prior to treatment for in-line treatments or as the ballast tank is filled for an in-tank treatment test cycle. The surrogates may also be added to large volumes of challenge water prior to treatment in a batch approach. The Test Plan will specify the approach selected. Biological efficacy tests shall only be conducted if indigenous populations are equal to or greater than the following threshold total abundances: 10^5, 10^4, and 10^2 cells per liter for total indigenous bacteria, total protists and total zooplankton, respectively[11]. Biological testing for treatment efficiency during the test cycles without surrogates may be conducted using the biological enumeration methods described below. *(This needs clarification.)*

[11] Rapid measurement approaches (i.e., epifluorescence for bacteria; screening and settling with rapid enumeration) for this test initiation threshold may be considered. Alternate methods must be described and justified in the Test Plan.

Due to the nature of the verification tests, a set of core parameters will apply to each Verification Test. Core parameters, sampling location, and sample/measurement approach are shown in Table 5-4.

Table 5-4. Core and Supplemental Parameters and Measurement Techniques

Parameter	Sample location and approach		Measurement location
	Challenge water	Post treatment	
Core Measurements			
Temperature	*In situ*, continuous	*In situ*, continuous	Test facility[12]
Salinity	*In situ*, continuous	*In situ*, continuous	Test facility
Total suspended solids	Discrete grab	Discrete grab	Laboratory
Particulate organic matter	Discrete grab	Discrete grab	Laboratory
Dissolved organic matter	*In situ*, continuous, discrete	*In situ*, continuous, discrete	Test facility, Laboratory
Dissolved oxygen	*In situ*, continuous	*In situ*, continuous	Test facility
Dissolved nutrients (N, P, Si)	NA	Discrete	Laboratory
Indigenous species	Discrete[a]	Discrete	Laboratory
Surrogate species	Discrete	Discrete	Laboratory
Proxy Measurements			
Turbidity (represents TSS)	*In situ*, continuous	*In situ*, continuous	Test facility
Chlorophyll a (biomass)	*In situ*, continuous	*In situ*, continuous	Test facility
ATP (living material)	Discrete grab, continuous as available	Discrete grab, continuous as available	Laboratory Test facility

[a] Depending on the species, the discrete sample for indigenous and surrogate species may be taken from the whole water grab sample or a volume of filtered water, usually through a net of specific mesh size.

Sampling for Verification Testing shall proceed under the standard flow conditions described under Section 5.3.3. For in-line treatments, samples will be collected during testing from locations just before and after the treatment unit or after the holding time. The samples will be simultaneously collected, time-integrated triplicates of 1 m^3 each obtained as the water enters or

[12] *In situ* measurements will be obtained from within ballast water flow lines of the treatment facility or simulated ballast tanks depending on the treatment approach.

exits the treatment unit or ballast tank after a holding period. Sub-samples for the core parameters will be obtained from this composite 1 m^3 sample, as described below. A single analytical replicate will be drawn from each composite tank.

Sampling Locations

Required sample locations for various treatment scenarios are shown in Figures 5-2, 5-3, and 5-4; samples must be collected according to one of these three test designs. Samples (data) from the challenge water must be obtained immediately prior to water entry into the treatment system, or ballast tank in the case of in-tank treatments. Samples of treated water must be collected immediately upon the water exiting the treatment system for in-line treatment systems, at the end of the Vendor defined contact period for in-tank treatments, and at the end of the holding times as described in Section 5.6. Sampling locations for the control tanks and system must exactly mimic the treatment tanks and system. The exact locations, frequency, and methods to be used to collect the samples must be defined in the Test Plan.

Sample Collection Requirements - Frequency

Continuously recording *in situ* sensors (as available and feasible) may be used to measure water quality and proxy parameters during Verification Testing. Description of the sensors, how they operate, and how they are calibrated shall be included in the Test Plan. Minimum instrument performance requirements are provided in Table 5-5 and Table 5-6. Discrete samples for water quality characterization will also be obtained during Verification Testing as discussed above, and will be collected at the time biological verification samples are collected. A higher frequency of collection for discrete samples may be used if additional samples for calibrating the sensors are necessary. The sample collection requirements and frequency of obtaining samples from the control tanks and system will identically match those of the treatment tanks and system. The appropriate frequency of discrete sample collections made in lieu of *in situ* sensing shall be described in the Test Plan.

Table 5-5. Accuracy and Precision Requirements for Sensors

Sensor	Reporting Units	Range	Accuracy	Precision
Temperature	ºC	0 to +30	0.01	0.01
Conductivity (salinity)	mS cm^{-1}	0.5 to 65	0.02	0.01
Transmissometer (20-cm)	m^{-1}	0 to 40	0.20	0.01
Dissolved oxygen	mg L^{-1}	0 to 20	0.10	0.05
Fluorometer	µg L^{-1}	0.03 to 75	50% of reading*	0.01

*When compared to wet chemistry results.

Table 5-6. Data Quality Objectives for Water Quality Samples

Core Parameter	Frequency of QC Sample Collection	Method Detection Limit	Data Quality Indicator Type/Acceptance Criteria
Dissolved nutrients	Procedural blank Two (2) per treatment cycle Sample replicates Three (3) sample duplicates per treatment cycle	*Ammonia and silica 0.02 μM* Nitrate, nitrite, phosphate 0.01 μM	Procedural blank <5 times MDL[1] Sample replicates ≤2% PD[2]
Total suspended solids (DI water and seawater)	Procedural blank Two (2) per treatment cycle Sample replicates Three (3) sample duplicates per treatment cycle	0.1 mg/L	Procedural blank <5 times MDL Sample replicates <10% RPD
DOC	Procedural blank Two (2) per treatment cycle Sample replicates Three (3) sample duplicates per treatment cycle	20 μM	Procedural blank ≤15% PD Sample replicates ≤10% RPD
POC	Procedural blank Two (2) per treatment cycle Sample replicates Three (3) sample duplicates per treatment cycle	5.5 μM	Procedural blank ≤15% PD Sample replicates ≤10% RPD
Chlorophyll *a*/phaeophytin	Procedural blank Two (2) per treatment cycle Sample replicates Three (3) sample duplicates per treatment cycle	0.02μg/L	Procedural blank ≤5% PD Sample replicates ≤15% RPD
Dissolved oxygen	Procedural blank NA Sample replicates Three (3) sample triplicates per treatment cycle		Procedural blank NA Sample replicates ≤5% CV[4]

[1] MDL = method detection limit
[2] Percent Difference (PD) = [(true concentration – measured concentration)/true concentration] × 100%.
[3] Relative Percent Difference (RPD) = [(absolute value (replicate 1 - replicate 2) □ 2/(replicate 1 + replicate 2)] × 100%.
[4] Filter blanks used for QC purposes only

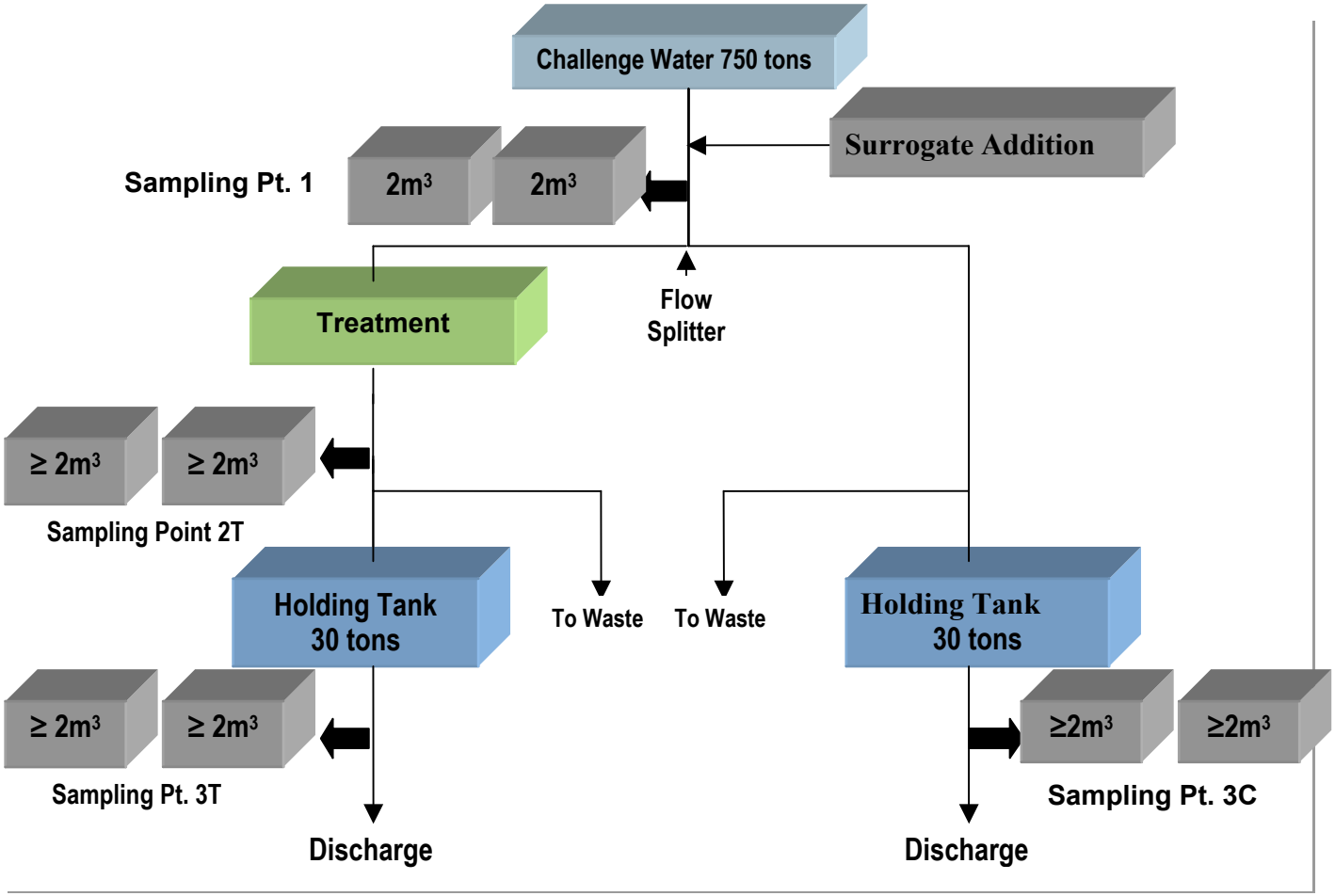

Figure 5-2. Sampling design for in-line treatment.

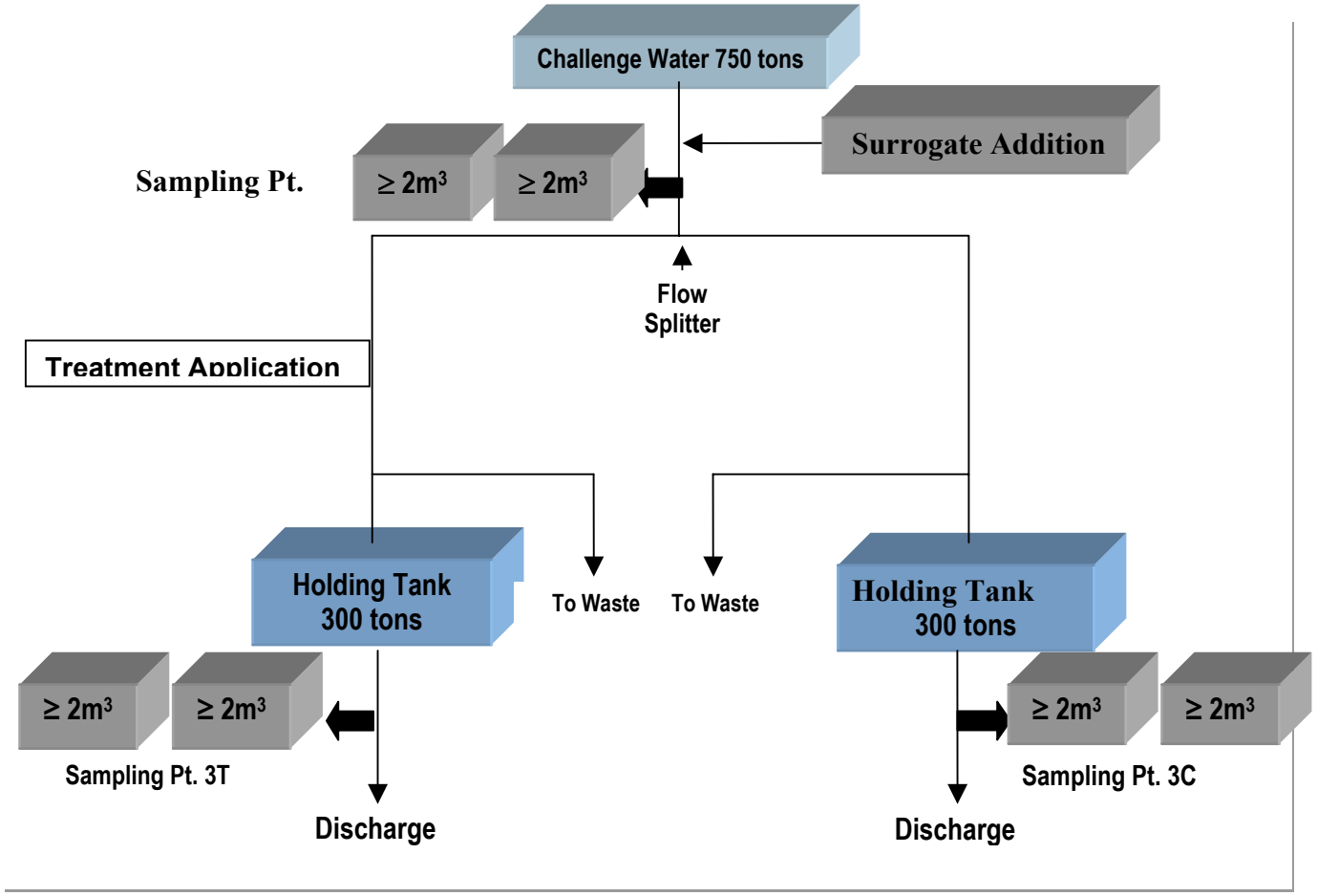

Figure 3-4. Schematic of sampling design for in-tank treatment.

Replication

Verification Testing will include three types of replication: 1) verification cycles, 2) biological treatment efficiency during a given test cycle, and 3) sampling and analysis. A minimum of six valid verification cycles will be required for the testing to be considered valid. Similarly, biological efficiency and associated variability are reportable results based on the six verification cycles and surrogate species used by the testing. Sample collection replicates are based on the integrated 1-m^3 triplicate samples collected at three points during the treatment cycle (see Figures 5-2, 5-3, and 5-4). These replicate samples form the minimum sample collection replication required during each Test cycle. Each of these integrated sample tanks will be sub sampled for the core parameters. Recommended quality control replicates and acceptance criteria for these analytical measurements are provided in Table 5-6. The Test Plan will describe each type of analytical replication planned including acceptable ranges of variability.

5.7.1 Biological Parameters

Biological samples will be collected using methods and techniques appropriate to the species group being measured. Guidance on sampling methods, sample volumes, sample container type, preservation method, holding time, analytical method reference are shown in Table 5-7. The testing organization will ensure that the contents of the integrating 1-m^3 sample collection tanks have been thoroughly mixed prior to sampling to ensure homogeneity.

Collection of water quality samples during stages of a multiple stage treatment process is optional. Inclusion of any additional samples must be described and justified in the Test Plan. Such data would be informational and not included as part of the Verification Data, but may be included as additional data in the Verification Report.

The Testing Organization, in conjunction with the Test Facility and the Vendor, will assess use of continuous, *in situ* (online) biological or other process measurements during verification testing. Any selected methods must be described and justified in the Test Plan, and approved for use by the Verification Organization.

The Testing Organization shall present a detailed schedule for Verification Test sample collection and analytical methods in the Test Plan. At a minimum, the Test Plan shall contain the scheduled sample collection times (expressed as time from start of test), parameters for testing, number of replicates, and number of control samples.

5.7.2 Supplemental Parameters

Sampling and analysis of supplemental parameters may be required depending on Vendor-specified information. For example, a Vendor may define an additional treatment effectiveness based on removal of fecal coliform. In those cases, the Testing Organization will determine the appropriate supplemental parameters, based on Vendor-specific information, and shall determine sampling and analysis requirements for inclusion in the sample collection schedule in the Test Plan.

Table 5-7. Sample Volumes, Containers, and Processing

Parameter	Min. Sample Volume (mL)[a]	Sample Containers[c]	Processing/ Preservation[c]	Maximum Holding Time
Electronic *in situ* data	NA	NA	Record data to floppy diskette.	NA
Dissolved inorganic nutrients	40	60-mL polyethylene bottle	Pass through a Nucleopore membrane filter; freeze filtrate until analysis.	28 days
Dissolved organic carbon	25	40-mL glass vial	Pass sample through a GF/F; freeze filtrate until analysis.	28 days
Particulate organic carbon	10 – 500 (500)	Whatman GF/F in foil	Pass through a GF/F; freeze filter until analysis.	28 days
Chlorophyll *a* and phaeopigments	25 – 400 (400)	Whatman GF/F in foil	Pass through GF/F; fix with a saturated $MgCO_3$ solution; freeze filter until analysis.	4 weeks
Total suspended solids	100 – 500 (300)	1-L dark bottle	Process immediately or store in 1-L dark bottle at 4°C for filtration.	1 week if stored
Dissolved oxygen	300	300 mL glass BOD bottle	Fix per Oudot *et al* (1988); titrate 2-24 h later.	24 hours
Bacterial – enumeration		Sterile plastic		
Phytoplankton – enumeration	850	1000 mL HDPE bottle	Preserve with Utermöhl's solu-tion; enumerate using standard plankton counting methods.	6 months
Zooplankton – enumeration	800 (100-L through screen)	1000-mL HDPE bottle	Quantitatively process water through 0.55 μ screen; wash into sample jar with screened seawater; fix with formalin after withdrawing viability sub-sample; store in dark at ambient temperature until analysis .	6 months
Bacterial - enrichment	(1000)	Sterile plastic	No preservation; transfer 20-μL aliquots into enrichment wells of tissue culture cells.	Process immediately; examine for growth 1-week after initiation sung plating techniques.
Phytoplankton - enrichment[d]	(1000)	Dark 1000-mL HDPE bottle	No preservation; transfer 100-μL aliquots into sterile styrene enrichment wells of tissue culture cells.	Process immediately; examine 1 and 2 weeks after initiation for growth.
Zooplankton – viability	5-mL aliquot of enumera-tion sample before preservation	10-mL glass or HDPE vial	No preservation; examine entire aliquot under dissecting microscope for organisms; observe and probe organisms to determine live/dead status.	Process immediately

GF/F: pre-ashed glass fiber filter
[a]Volume processed for analysis; volumes are quantitative.
[b] Conductivity, temperature, pressure, dissolved oxygen, chlorophyll a fluorescence, transmissometry
[c]Name brand items (*e.g.*, Nuclepore, Whatman) may be substituted with comparable items from a different manufacturer.
[d]Dinoflagellate methods are under development

5.7.3 Analytical Methods

Water Quality

Recommended methods for water quality analysis are listed in Table 5-8**.** Reliable, continuously recording *in situ* sensors are available for temperature, salinity, dissolved oxygen, particulate organic matter (i.e., measures of solids concentrations such as nephelometry or transmissivity), dissolved organic matter (i.e., as fluorescence from color forming compounds), and chlorophyll. Such sensors may, with Verification Organization approval, be used to measure water quality parameters during verification testing. Discrete analytical samples shall be collected to provide test specific verification or calibration of the sensor data and to allow comparison of sensor data to Vendor supplied information as appropriate. Sensor maintenance and calibration shall be described in the test site operating procedures and/or the Test Plan.

Table 5-8. Core Parameter Methods

Parameter	Units	Instrument	Method/Reference
Discrete Samples			
Dissolved ammonium	µM	Autoanalyzer	ASTM Method No. D1426-03 APHA Standard Method No. 4500-NH3 , 20th edition EPA Method No. 349.0 http://www.epa.gov/nerlcwww/m349_0.pdf; Oviatt and Hindle (1994); Solorzano (1969)
Dissolved inorganic nitrate and inorganic nitrite	µM	Autoanalyzer	ESS Method No. 220.3 (LMMB Method No. 061) http://www.epa.gov/glnpo/lmmb/methods/methd220.pdf ASTM Method No. D3867-99 APHA Standard Method Nos. 4500-NO2-B and 4500-NO3-F, 19th edition EPA Method No. 353.4 http://www.epa.gov/nerlcwww/m353_4.pdf Bendschneider and Robinson (1952), and Morris and Riley (1963)
Dissolved inorganic phosphate	µM	Autoanalyzer	ESS Method No. 310.1 (LMMB Method No. 063) http://www.epa.gov/glnpo/lmmb/methods/methd310.pdf EPA Method No. 365.5 http://www.epa.gov/nerlcwww/m365_5.pdf Murphy and Riley (1962)
Dissolved inorganic silicate	µM	Autoanalyzer	EPA Method 366.0 http://www.epa.gov/nerlcwww/m366_0.pdf Brewer and Riley (1966); Oviatt and Hindle (1994)

Table 5-8. Core Parameter Methods (continued)

Parameter	Units	Instrument	Method/Reference
Dissolved organic carbon	µM	Carbon Analyzer	APHA Standard Method No. 5310-C, 20th edition ASTM Method Nos. D6317, D2579, D4129, D4839, D513-02 and D5790 SW846 Method No. 9060 http://www.epa.gov/epaoswer/hazwaste/test/pdfs/9060.pdf LMMB Method No. 096 http://www.epa.gov/glnpo/lmmb/methods/docanal2.pdf LMMB Method No. 014 http://www.epa.gov/glnpo/lmmb/methods/pocdoc2.pdf EPA Method No. 440.0 http://www.epa.gov/nerlcwww/m440_0.pdf Sugimura and Suzuki (1988)
Particulate organic carbon	uM	Carbon analyzer or CHN Analyzer	LMMB Method No. 097 http://www.epa.gov/glnpo/lmmb/methods/pocanal2.pdf APHA Standard Method No. 5310-C, 20th edition LMMB Method No. 014 http://www.epa.gov/glnpo/lmmb/methods/pocdoc2.pdf EPA Method No. 440.0 http://www.epa.gov/nerlcwww/m440_0.pdf
Chlorophyll a/phaeopigments	µg/L	Fluorometer	EPA Method 445.0 http://www.epa.gov/nerlcwww/m445_0.pdf EPA Method No. 446.0 http://www.epa.gov/nerlcwww/m446_0.pdf EPA Method 447.0 http://www.epa.gov/nerlcwww/m447_0.pdf ASTM Method No. 3731-87 (1998)
Total suspended solids	mg/L	5-place balance *Note: glass fiber can not be used for seawater TSS determinations due to salt retention; membrane filters are required.*	ESS Method No. 340.2 (LMMB Method No. 065) http://www.epa.gov/glnpo/lmmb/methods/methd340.pdf LMMB Method No. 090 http://www.epa.gov/glnpo/lmmb/methods/turbid.pdf APHA Standard Method No. 2540D (1998) EPA Method 160.2 http://www.epa.gov/region09/qa/pdfs/160_2.pdf
Mineral matter	mg/L	Difference between TSS and Particulate Carbon measures	NA
Dissolved oxygen	mg/L	Radiometer TitraLab	ASTM Method No. D5543-94 ASTM Method No. D5462-02 ASTM Method No. D888-92 EPA Method No. 360.1 (Probe Method) http://www.umass.edu/tei/mwwp/acrobat/epa360_1Doprobe.pdf APHA Standard Method No. 4500-0G (Probe Method) Oudot *et al.* (1988)
Microbial – enumeration	TBD	TBD	TBD

Table 5-8. Core Parameter Methods (continued)

Parameter	Units	Instrument	Method/Reference
Phytoplankton –enumeration	E6Cells /L	Microscope with phase-contrast optics or an inverted scope	Method No. LMMB 023 http://www.epa.gov/glnpo/lmmb/methods/phytocol.pdf Borkman (1994), Borkman *et al.* (1993), Turner *et al.* (1995)
Zooplankton - enumeration	Indiv./ m^3	Dissecting microscope	Method No. LMMB 024 http://www.epa.gov/glnpo/lmmb/methods/zoofld.pdf
Enrichment technique – Bacteria	TBD	Tissue culture cells; bacteria plating media; or epifluorescence microscope	Modified from Rogerson and Gwaltney (2000) and Hall *et al*. (1996);
Enrichment technique – protists	TBD	Tissue culture cells and microscope or epifluorescence microscope for direct counting	Modified from Rogerson and Gwaltney (2000) and Hall *et al.* (1996);
Zooplankton technique - viability	TBD	Dissecting microscope	Observe and probe to determine movement
***In situ* Measurements**			
Temperature	ºC	CTD	Instrument Manual
Salinity	PSU	CTD	CTD Manual
Dissolved oxygen	mg/L	DO probe	Probe Manual.
Chlorophyll fluorescence	µg/L	Fluorescence sensor	Instrument Manual
Transmissometry/ Nephelometry	m^{-1}/ NTU	Transmissometer/ Nephlometer	Instrument Manual/ Instrument Manual

Discrete samples for determination of total suspended solids, particulate organic matter (as carbon), total dissolved organic matter (as DOC), and nutrient concentrations shall be collected appropriate to the tests being conducted. The concentration of mineral matter may be determined as the difference between the total suspended solids and the particulate organic matter concentration (mass per liter basis). The total suspended solids determination in seawater shall not use glass fiber in the filtration step due to false positives caused by salt retention on glass fiber filters. Membrane filters such as Nucleopore™ or other similar type shall be used for measurement of total suspended solids determinations in seawater

The analytical methods must be applied within defined holding times (Table 5-7) after appropriate preservation, per industry standard procedures. Where available, USEPA, *Standard Methods* or other methods approved by the Verification Organization (i.e., ASTM) will be used to quantify each parameter. If standard methods are not available, the sampling and analytical methods to be used shall be documented in the Test Plan. These methods will follow accepted scientific practices and be accepted by the Verification Organization.

The Testing Organization, in consultation with the Verification Organization and Vendor, may propose other parameters and methods. The methods shall be documented in the Test Plan.

Biological Organisms

The abundance and viability of the indigenous and surrogate species will be quantified in the challenge water before and after each verification cycle. Recommended measurement methods are listed in Table 5-8 and are described in the following sections.

Bacteria - Sample analysis will be conducted according to standard microbial techniques[13]. Multiple bacterial growth media will be used to assess the effectiveness of a treatment for bacteria[14]. Use of multiple types of media enables measurement of the response of different portions of the indigenous bacterial community[15]. The minimum number of media used will include two general-purpose media for heterotrophic bacteria, a marine agar, and a nutrient agar. Other media may be added during the development of the Test Plan. The rationale and methods will be described in the Test Plan.

Appropriate controls (e.g., heat to remove vegetative cells for tests using resting stages or spores) for microbial plates will be used throughout the Verification Testing. Steps will also be taken to ensure the action of any treatment (e.g., a biocide) is stopped at the time of sample collection (i.e., treatment does not continue after sample collection). Any steps and controls used to verify step will be described and justified in the Test Plan.

Protists – Enumeration Technique **-** The preserved sample will be prepared for analysis by concentrating the sample. One method commonly used is gravitational settling as described by Borkman (1994), Borkman *et al.* (1993), Turner *et al.* (1995). The method is similar to the methods of Hasle (1959), Iriarte and Fryxell (1995), and Sukhanova (1978). In this method, organisms are settled in graduated cylinders with no more than a 5-to-1 height-to-width ratio. The species are observed and counted using a phase contrast microscope.

For the settling approach above, protist abundance is quantified by counting the phytoplankton cells in a 1-mL capacity Sedgwick-Rafter chamber using a compound microscope with phase contrast[16]. Phytoplankton cells will be observed, counted, and identified in a two-stage counting protocol utilizing 250× and 500× magnifications. In this protocol, the Sedgwick-Rafter chamber is divided into equal, horizontal paths or strips and cells are enumerated as one moves across randomly selected strips. Small cells (*e.g.*, microflagellates, *Cryptomonas*) will be counted at 500×, with counting of small cells proceeding at 500× until the end of the path in which the 400-

[13] Standard methods are being identified

[14] The suggested media for marine water include 2216 Marine Agar and salt-modified R2A agar; media for fresh water species may include Plate Count Agar (plus 2% NaCl); Nutrient broth (plus agar (15 g/L) plus 2% NaCl).

[15] Note it is assumed that if all culturable bacteria are killed all non-culturable bacteria are also killed.

[16] Other comparable methods may be used to enumerate the organisms. However, methods and materials must be documented in the Test Plan.

entities minimum tally is reached[17]. The analysis will continue at 250× with paths of the Sedgwick-Rafter chamber being examined until, when practical, at least 75 entities (unicellular forms, colonies, or chains) of each of the three most abundant taxa are observed.

The two-step counting protocol allows for improved precision in estimating abundances of small (<10μm greatest axial linear dimension) and larger phytoplankton forms. Counting large numbers of small forms at 500× increases the precision of the estimated abundances of these forms (see Section 11 for a discussion of precision). The counts at 250× allow for the examination of a larger volume of the sample, thereby increasing the likelihood of encountering larger, less abundant (or rare) forms. During the 250× analysis, the 500× objective can be used as needed to resolve key taxonomic characters.

The theoretical maximum volume that would be examined is an entire Sedgwick-Rafter cell (1 ml). Typical volumes are one path of the cell, which at 500× equals 1/48 of one ml of concentrate, and at 250× equals 1/24 of one ml of concentrate. The volume of sample that is examined is dependent on number of cells encountered and how long it takes to reach cut-offs of 75 entities of the top 3 taxa, 400 cells total. Volumes processed are 800 ml of water, settled to 50 ml of concentrate, for a 16:1 ratio. Final abundance estimates will be reported in cells per liter (normally this is in units of 10^6 cells per liter. Total abundance (sum of all species counted) will be used in statistical tests.

Other methods may be used to concentrate organisms with visualization of the organisms for enumeration using an inverted microscope.

Protists – Enrichment Technique - The enrichment technique (Rogerson and Gwaltney, 2000; Hall *et al*., 1996) will include a dilution series sufficient to ensure changes of at least 10^5 can be detected under the protists viability test. Isolation and viability testing of single resting cells of protist surrogates should be attempted. Protists will be tested under light and dark testing conditions. This enables evaluation of the response of autotrophic (light) and heterotrophic (dark) organisms. The heterotrophic branch of the enrichment measurement includes such organisms as amoebae, ciliates, nanoflagellates, (2-20 μm) and other dinoflagellates. The autotrophic branch of the test evaluates algae (i.e., organisms such as diatoms, autotrophic nanoflagellates, other flagellates, and non motile algae). Light and dark incubations will include media appropriate to the specific organism type and will include different levels of nutrient in the media.

The measurement approach will include the dilution series by media and incubation at a constant temperature for 7 to 14 days. A +/- scoring of each enrichment sample will provide the data to

[17] Based on Guillard (1973), counts of 400 phytoplankton cells will provide a precision of ±10% of the mean. For this program, a minimum of 400 entities (solitary single cells, chains, or colonies) will be tallied for each sample. Unicellular forms (*e.g.*, *Cryptomonas*, microflagellates), aggregate forms (*e.g.*, *Phaeocystis*), and chained forms (*e.g.*, *Skeletonema*) will each count as one entity towards the 400-entities-counted-per-sample minimum tally. To increase precision of the abundance estimates for the most abundant taxa, when practical at least 75 entities of each of the three most abundant taxa will be counted in each sample. The overall goal then is to enumerate the three most abundant taxa to at least 75 entities each, and enumerate 175 entities (400-3*75) of other taxa.

evaluate the response. The enrichment analysis should be set up to measure a 1 in 100,000 change. (Rogerson and Gwaltney, 2000; Hall *et al*., 1996).

Zooplankton Enumeration - Preserved zooplankton samples shall be rinsed and transferred to 70 percent ethanol solutions to prevent inhalation of formalin fumes during sample counting. Samples shall be reduced to aliquots of at least 300 hundred animals with a Folsom plankton splitter. The animals in one of the aliquots shall be counted under a dissecting microscope to the lowest possible taxon. In most cases, this shall be to species. Counts of all copepodite stages of a given copepod genus shall be combined. Copepod nauplii shall not be identified to genus or species because nauplii cannot be reliably identified to those levels by using a dissecting microscope. Meroplankters cannot be identified to genus or species in most cases, and such organisms shall be identified to the lowest reliable taxon, such as barnacle nauplii, fish eggs, or gastropod veligers.

Abundance of all identified taxa and total zooplankton (sum of the individual species in a sample) shall be calculated based on the number of animals counted, divided by the volume of water filtered by the net, multiplied by an aliquot concentration factor. Total abundance (sum of all animals counted) will be used in statistical tests.

Toxicity Testing for Biocide Treatment Technologies

Toxicity tests conducted during the start-up if treatments involving biocides will be selected from the following:

Inland Silverside, *Menidia Beryllin*a, Larval Survival And Growth (EPA Method 1006.0): http://www.epa.gov/OST/WET/disk1/ctm13.pdf**)**

Sea Urchin, *Arbacia Punctulat*a, Fertilization Test (EPA METHOD 1008.0: http://www.epa.gov/OST/WET/disk1/ctm15.pdf)

Mysid Acute Toxicity Test (EPA OPPTS Method 850.1035: http://www.epa.gov/opptsfrs/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Drafts/850-1035.pdf)

Additional guidance can be found in ASTM (1996) *Standard Guide for Conducting Acute Toxicity Tests Starting with Embryos of Four Species of Saltwater Bivalve Mollusks*. Annual Book of ASTM Standards, Section 11, Water and Environmental Technology, Volume 11.05 (724-94), ASTM (1996) *Standard Guide for Conducting Acute Toxicity Tests with Echinoid Larvae", Section 11, Water and Environmental Technology*, Volume 11.05 (E1563-95), and Donald J. Klemm, George E. Morrison, Teresa J. Norberg-King, William H. Peltier, and Margarete A. Heber (1994) *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms* (Second Edition) EPA/600/4-91/003.

Tests selected for use during the start-up testing will be specified in the Test Plan. At least one freshwater and one salt-water species shall be specified and tested.

Proxy Measures

Continuous sensing of chlorophyll biomass and turbidity will be conducted with continuous, *in situ* sensors. Fluorescence measurements will be calibrated using extracted chlorophyll samples collected and analyzed based on USEPA or ASTM Standard Methods (see Table 5-8 for methods). Specific methods shall be described in the Test Plan. Calibrated chlorophyll responses will be reported in the verification report.

5.8 Operation and Maintenance Verification Factor

As part of the testing, the operation and maintenance (O&M) of the ballast water treatment system will be verified. The verification has been designed so that minimum test duration is not less than 150 percent of the Vendor's recommended O&M cycle, or as a minimum volume requirement, therefore allowing sufficient time to verify operation and maintenance of the ballast water treatment system.

The Testing Organization is responsible for monitoring and maintaining the system throughout the duration of the testing to ensure stable operating conditions (as mutually agreed to by the Vendor) and proper operating effectiveness. All system components will be monitored for proper operation throughout the test period. Equipment and components will be maintained in accordance with the O&M manual provided by the Vendor. All maintenance activity completed during the verification testing shall be documented for inclusion in the Verification Report.

All required monitoring and maintenance activities should be coordinated with the Testing Organization in advance of verification testing, and detailed in a monitoring and maintenance plan included in the Test Plan. The monitoring and maintenance plan shall address the following requirements, as applicable:

- A monitoring and maintenance schedule for the testing period. The experimental design for verification calls for verification testing duration of no less than 150 percent of the vendor-specified O&M cycle. This allows frequent system inspection, and for at least one formal scheduled system maintenance.
- Equipment and component calibration methods and frequencies.
- Monitoring and maintenance activities and procedures shall be described and documentation forms provided. Maintenance documentation forms must identify the Test Facility, date, and time; describe the work performed, observations of the treatment system, and results of the work.
- Operating characteristics and Vendor-specified ranges required for proper operating conditions shall be described (e.g., system temperature, flows entering and exiting the system, power levels).

Other information that must be addressed in the Test Plan includes:

- Monitoring requirements to ensure a proper operating environment;
- Continuous on-line O&M monitoring requirements, as specified by the Vendor; and,

- Credentials of all personnel involved in operating, monitoring and maintaining the treatment system.

All monitoring and maintenance documentation must be maintained in a written record at the Testing Facility and will be included in the Verification Report.

To help address predictability and reliability verification factors, qualitative and quantitative O&M performance indictors will be evaluated. The means and methods to evaluate or quantify O&M performance indicators shall be included in the Test Plan, and described in a schedule for collecting this information.

5.8.1 Qualitative O&M Performance Indicators

Qualitative O&M performance indicators will include, but are not limited to:

- *Visual Observations* - Visual inspections of the treated ballast water quality (e.g., turbidity, color) and treatment technology conditions (e.g., foaming, floating material, settled solids) will be performed at each maintenance or monitoring event. Visual observations will also include the inspection of the treatment system prior to, during and following each test cycle for equipment and process failures, corrosion, leaks, impediments of flow (entering or exiting the system) and any other system issues that could impact performance. Specific visual indicators shall be defined in the Test Plan.
- *Operability* - Observations regarding the ease of start-up and operation during testing and the ease of monitoring system performance shall be noted and recorded.
- *O&M Manual* - The Testing Organization shall evaluate the usefulness and quality of the O&M manual, and a written report on the evaluation shall be prepared.
- *Operator Skills* - The level of operator expertise required to operate and maintain the treatment technology shall be noted.
- *System Accessibility* - The ease of access and required clearances for system operation and required maintenance shall be noted.

5.8.2 Quantitative O&M Performance Indicators

Quantitative O&M performance indicators shall include, but are not limited to:

- *Time demand* - Personnel time required to start-up, shutdown, operate, and maintain the treatment technology shall be recorded in the monitoring and maintenance log.
- *Residuals* - Volumes of residual materials, (e.g., solids removed via filtration systems, etc.), mass generation rates and concentrations shall be measured during verification testing. Results will be recorded in m^3, gallons or pounds per m^3, or gallons of water treated, as appropriate. Factors related to the disposal of residuals (such as storage requirements and handling hazards) shall also be addressed.
- *Chemical Use* - Usage rates and concentrations of any chemicals (e.g. biocides) used as part of the treatment system and its operation during verification testing (per test cycle) will be measured and recorded. Results shall be reported as for residuals.

- *Power consumption* - The power consumed per test cycle by the treatment technology will be monitored and recorded (e.g. kWh per m^3 of water treated shall be calculated). The peak electrical load at system start-up will also be monitored and recorded, as will fluctuations in consumption during test cycles.
- *Other Consumables* - The use of any other consumables, such as filter cartridges, shall be monitored, documented, and reported.

5.8.3 Supplemental Parameters

Depending on Vendor claims, supplemental monitoring, maintenance, and O&M performance indictors may be required. These will be described, along with requirements for performance monitoring, in the Test Plan.

5.8.4 Upset Conditions

The Testing Organization shall notify the Vendor and the Verification Organization immediately when an upset condition is identified. The Testing Organization shall correct the upset condition as soon as possible to bring the treatment system back on line. For unusual upset conditions, the Testing Organization will work with the Vendor to identify and correct the problem. The occurrence of all upset conditions, the causes, the results, and the means to correct the upset shall be documented in the Verification Report.

Results of sampling performed during upset conditions shall not be included in the statistical analysis for the Verification Report, but shall be identified and discussed in the Verification Report. If the cause of an upset condition cannot be determined or the condition cannot be qualified as a true upset, then the sampling results shall be used in the statistical analysis for the Verification Report.

5.9 Reliability

The reliability of the treatment system will be determined by 1) the number of instances where the treatment system or technology does not achieve the stated performance goal per the total number of test cycles, and 2) the standard deviation of the mean for biological performance data (e.g., percent removal).

Reliability performance measures will take into consideration any vendor provided information that assists in the projection of the performance such as CT (concentration X time) or power/energy curves. Any adjustments made to the system, outside of the vendor-specified operation and maintenance claims, to achieve the performance goals will be noted in the maintenance log and specified in the Verification Report.

Specific performance reliability indicators along with the planned methods for evaluating and reporting them will be identified in the Test Plan.

5.10 Cost Factors

Verified cost factors will include the following as applicable:

- *Power consumption* – reported as total kWh necessary to operate all equipment to achieve desired biological treatment performance.
- *Consumable or expendable materials* – amounts of for all consumables or expendables, including chemicals or other items required for treatment shall be itemized and reported.
- *Replacement parts used during normal maintenance* – number of replacement parts will be itemized and reported. Any unanticipated replacement parts will be specified separately.
- *Labor time to start-up, operate, and maintain the treatment system* – total number of hours for each activity will be recorded and reported.
- *Byproduct or waste materials produced* – which require treatment or disposal will be reported as an expression of total volume treated or disposed.

5.11 Environmental Acceptability

Two performance indicators will determine the Environmental Acceptability of a treatment system: Water Quality and Treatment Residuals.

The data used to evaluate the environmental acceptability of a system will be taken from the water quality data collected at the point of discharge as detailed in Section 5.7. This data will include but may not be limited to the following parameters:

- Temperature
- pH
- Salinity
- Total suspended solids
- Particulate organic matter
- Dissolved organic matter
- Dissolved oxygen
- Dissolved nutrients
- Biological oxygen demand

The results of these tests at the point of discharge will be compared to the range of expected natural conditions and reported in the Verification Test.

Additional analytical parameters will be included as necessary for reporting on any residual material that may result from treatment; for example residual biocides and disinfection byproducts. The additional parameters, the potential impact to the environment, and the analytical methods will be detailed in the Test Plan

It will be the responsibility of the Testing Facility to obtain NPDES discharge permits and to ensure that discharge is within permitted limits. However, toxicity testing of any biocide treatment will be conducted, as discussed under Section 5.4.3. Verification testing will not begin unless the results of the toxicity tests are acceptable.

5.12 Safety

Safety is of concern during the operation of any equipment or machinery and during the use of potential hazardous materials, but of particular concern while on board ship, where staff is

limited and access to land based emergency infrastructure is unavailable. Therefore, the safety of the treatment system will be evaluated during verification testing.

The performance indicators for this verification factor will be technology specific. However, required indicators shall include, to the extent possible:

- Listing of all dangerous or hazardous materials, including submittal of Material Safety Data Sheets (MSDS).
- Potential to compromise the normal ship ballasting or deballasting cycle (i.e., impediment of flow).
- Visual indicators of potential threats to shipboard operations, such as exposed or improper housing of power cables, structural stability of the system, external temperatures of the of the treatment system, and any other treatment-specific factors that may pose a threat to the operator or compromise the safety of ship operations.
- Review of the Vendor provided O&M manual for adequacy of cautions and guidance on ways to minimize the potential for, and directives to mitigate, a hazardous situation.

The method for evaluating these and other items identified by the Testing Organization in reviewing the technology documentation shall be described in the Test Plan.

Chapter 6
Reporting Verification Testing Results

All testing results will be presented in a Verification Report, which will include all data regarding challenge conditions, results of verification testing for all verification factors, and any vendor supplied data or information. A summary Verification Statement will also be prepared, presenting the most important results of the verification testing.

The outline for the report shall include:

- Verification Statement
- Executive Summary
- Introduction and Background
- Description of the Treatment Technology or System
- Description of Challenge Conditions
- Experimental Design
- Methods and Procedures
- Results and Discussion
- Verification Testing Operation and Monitoring QA/QC

Appendices:

- Test Plan
- Vendor-supplied Operation and Maintenance Manual
- Data Generated During Testing
- QA/QC Records
- Maintenance Logs
- Any other records maintained during testing
- Any other information provided by the vendor, which may be of use to the stakeholder community.

Upon completion of the draft report the Verification Organization, the Vendor, and the Test Facility QA Manager will review the document and provide comment. The comments will be incorporated or stricken with approval of all parties and the Final Report will be submitted to NSF International (ETV Water Quality Protection Center Partner) and EPA for approval.

Chapter 7
Test Facility Requirements

The Test Facility will have the following general specifications to adequately conduct the verification testing in accordance with the standards set by the ETV Program and the testing requirements described in this protocol.

Note: These requirements are under development and subject to revision.

- Challenge water from fresh to salt water at flow rates of up to 300 m^3 per hour (1,320 gal per minute) for both treatment and control streams for up to two (2) hours duration (available volume per test cycle up to 1200 m^3) per test cycle.
- Ability to perform at least one test cycle per day and facilities to hold water after treatment (i.e. holding tanks of up to 600 m^3 [assumes only half of the flow diverted to holding tanks for treated volumes greater than 300 m^3 per hour).
- Ability to conduct in-line and in-tank treatment verifications simultaneously.
- Ability to repeat biological tests within five working days of previous cycle.
- Surrogate species assay faculties.
- Sampling capability up to 18 onsite 1-m^3 triplicate sampling tanks per treatment type (up to 24 if can run in-line and in-tank simultaneously) Assumes one of the sets is reused for holding tank sampling.
- Analytical laboratory facilities for enrichment techniques and zooplankton viability testing.
- Analytical laboratory facilities for discrete sample analysis (DO, Chl, nutrients).
- *In situ* continuous sensing systems linked to data acquisition system.
- Facilities for measuring engineering performance.
- Per Section 5.7, the indigenous populations must have threshold abundances of 10^5 bacteria cells per liter, 10^4 total protists per liter and 10^2 zooplankton per liter.

Chapter 8
Quality Assurance/Quality Control (QA/QC)

To ensure the quality and integrity of data gathered during testing activities, a Quality Assurance Project Plan (QAPP) will be prepared by the Testing Organization and included as part of the Test Plan. The QAPP will describe the project scope, management, procedures for measurements and data acquisition, project assessment and oversight, and data validation and usability assessments necessary to meet the project goals. The written document will serve to communicate all decisions related to project design and completion to the project team so that work is performed according to written specifications. The generic format for a QAPP is included in Appendix A.

8.1 Verification of Test Data

The QAPP will address data quality in part by the development of acceptable values for six data quality objectives: accuracy, precision, completeness, comparability, representativeness, and sensitivity. The data quality objectives will establish the locations, types and numbers of samples to be collected, the quality control samples (duplicates, blanks, spikes, etc.) required for both field and laboratory samples, and will establish the data quality criteria and measures of acceptability that are appropriate for the project. The Test Plan will also detail a corrective action plan to describe actions to be taken if acceptance criteria are not met.

8.2 Project Management

The QAPP will list all project participants and clearly define their roles and responsibilities. In addition, this section will describe project scheduling, data quality objectives, training and certification requirements (as applicable), and required documentation. The information included in this section will ensure that all participants understand the scope of the study and their explicit roles.

8.3 Measurement and Data Acquisition

A detailed description of the experimental design and its components will be included in the QAPP. Specific requirements with regard to use, maintenance, and calibration of equipment, analytical procedures, chain-of-custody procedures, sample collection, data management and documentation, records management, project scheduling, experimental design assumptions, and disclosure of non-standard techniques or equipment will be discussed.

8.4 Assessment

The effectiveness of QA/QC will be monitored through assessments of general and project-specific activities. The QAPP will include detailed information on the types of assessments to be utilized (e.g., management, technical, and/or quality assurance assessments), appropriate response actions, reporting requirements, and assessment and reporting authority.

Chapter 9
Data Management, Analysis and Presentation

9.1 Data Management

Any data collected during testing activities must be capable of withstanding challenges to its validity, accuracy, and legibility. Data will be recorded in standardized formats and in accordance with the following minimum requirements:

- Data are entered directly, promptly, and legibly.
- Data are recorded legibly in ink. All original data records include, as appropriate, a description of the data collected, the unit, the unique sample identification, the name of the person collecting the data, and the date and time of data collection.
- Any changes to the original entry do not obscure the original entry, document the reason for the change, and are initialed and dated by the person making the change.
- All deviations from the QAPP must be documented in writing, and approved by the appropriate authority. Documentation and communication include an assessment of the impact the deviation has on data quality.
- Data in electronic format shall be included in commercially available program for word processing, spreadsheet, or database processing, or commercial software developed especially for the data collection and processing on a specific hardware instrument or piece of equipment. Backup of computer databases should be performed on a daily basis, if possible.

Project-specific data management requirements, including the types of data to be collected and managed, and how they will subsequently be reported, shall be defined in the data handling section of the Test Plan. QA/QC activities for data management will be described in the QAPP, included in the Test Plan.

9.2 Data Analysis and Presentation

Raw data gathered during verification testing will be entered into electronic format (spreadsheet or other database product capable of performing graphical and simple statistical analyses). Following reduction, data will be presented in a graphical, tabular, or other logical format, and accompanied by a detailed discussion to be included in the Verification Testing Report.

Treatment effectiveness will be calculated for each indigenous species and surrogate species as the percentage of the organism removed by the treatment ((Ci-Co)/Ci)*100), where Ci is the abundance on the input side and Co is the abundance on the output side of the treatment. The statistical significance of the removal will be evaluated relative to the treatment control using a t-test based on the mean of treatment efficiency based on the sample replicates after any analytical replicates are averaged. All methods will be described in the Test Plan. The treatment effectiveness will be discussed in the Verification Testing Report. Raw data will be included as an appendix to the Verification Testing Report.

Chapter 10
Environmental, Health, and Safety Plan

The Testing Organization shall develop an Environmental, Health, and Safety (EHS) Plan to be included in the Test Plan. The EHS Plan shall identify all environmental concerns and potential hazards associated with the verification testing process and the Test Facility, as well as the required measures to prevent exposure to them. The Testing Organization shall be responsible for informing all personnel at the test site, including employees, contractors, and visitors, of the potential hazards and safety measures to be employed at the test site. The EHS Plan shall address the following issues, as applicable:

- Permitting requirements for equipment operation, effluent discharge, and waste disposal.
- Biological, chemical, mechanical, electrical, and other hazards. Environmental hazards will be defined in accordance with local, state and federal regulations.
- Handling, storage, and disposal of all biological material and chemicals associated with the testing.
- Material Safety Data Sheets (MSDS).
- Conformance with the local electrical code.
- Conformance with the local plumbing code.
- Ventilation of equipment, trailers, or buildings housing equipment, if gases generated by the equipment could present a safety hazard.
- Confined space entry hazards.
- Fire safety.
- Emergency contacts for 911, the nearest hospital (provide directions), local fire department, the site manager, and all other important contacts.

OSHA Safety Devices

Any other environmental, health, or safety issues specific to the test location or ballast water treatment technology to be tested must be addressed. A copy of the EHS Plan, including all MSDS, shall be maintained and readily accessible at the test site. A one-page summary of emergency contacts shall be placed inside a clear plastic cover and kept at the verification-testing unit.

Chapter 11
References

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APPENDIX A

TEST PLAN REQUIREMENTS

A Quality Assurance Project Plan (QAPP) shall be prepared as part of the Test Plan for evaluating the performance of ballast water treatment technologies. The generic format for such QAPPs includes:

1.0 PROJECT DESCRIPTIONS, OBJECTIVES and ORGANIZATION

1.1 The purpose of the study shall be clearly stated.

1.2 The processes to be evaluated will be described.

1.3 The facility, apparatus and technology set-up will be fully described.

1.4 Project objectives shall be clearly stated and identified as being primary or non-primary.

1.5 Responsibilities of all project participants shall be identified. Key personnel and their organizations shall be identified, along with the designation of responsibilities for planning, coordination, sample collection, measurements (i.e., analytical, physical, and process), data reduction, data validation (independent of data generation), data analysis, report preparation, and quality assurance.

2.0 EXPERIMENTAL APPROACH

2.1 Technology installation and shakedown procedures will be identified.

2.2 Technology startup procedures will be identified. Startup will comprise a number of tasks to implement and check operating and sampling protocols. Tasks will include establishing feed makeup and performing calibration checks on monitoring systems, identifying sampling and monitoring points and identifying the types of samples to be collected.

2.3 Physical, analytical or chemical measurements to be taken during the study will be provided. Examples include flow rates, pH, salinity, total suspended solids, particulate organic matter, dissolved organic matter, dissolved oxygen, dissolved nutrients, biochemical oxygen demand, biological organisms, O&M performance indicators, etc.

2.4 Sampling and monitoring points for each test unit and the type of sample to be collected (grab or composite) will be identified.

2.5 The frequency of sampling and monitoring as well as the number of samples required will be provided. This includes the number of samples needed to meet QA/QC objectives.

2.8 Planned approach for evaluation objectives (data analysis). This will include formulas, units, and definition of terms and statistical analyses to be performed in the analysis of the data. Example graphical relationships will be provided.

2.7 Demobilization of the technology, including scheduling and site restoration requirements, will be described.

3.0 SAMPLING PROCEDURES

3.1 Whenever applicable or necessary to achieve project objectives, the method used to establish steady-state conditions shall be described.

3.2 Each sampling/monitoring procedure to be used shall be described in detail or referenced. If compositing or splitting samples is required, those procedures shall be described.

3.3 Sampling/monitoring procedures shall be appropriate for the matrix/analyte being tested.

3.4 If sampling/monitoring equipment is used to collect critical measurement data (e.g., used to calculate the final concentration of a critical parameter), the QAPP shall describe how the sampling equipment is calibrated.

3.5 If sampling/monitoring equipment is used to collect critical measurement data, the QAPP shall describe how cross-contamination between samples is avoided.

3.6 When representativeness is essential for meeting a primary project objective, the QAPP shall include a discussion of the procedures to be used to assure that representative samples are collected.

3.7 A list of sample quantities to be collected, and the sample amount required for each analysis, including QC sample analysis, shall be specified in the QAPP.

3.8 Containers used for sample collection for each sample type shall be described in the QAPP.

3.9 Sample preservation methods (e.g., refrigeration, acidification, etc.) and holding times shall be described in the QAPP.

4.0 TESTING AND MEASUREMENT PROTOCOLS

4.1 Each measurement method to be used shall be described in detail or referenced in the QAPP. Modifications to EPA-approved or similarly validated methods shall be specified.

4.2 For unproven methods, the QAPP shall provide evidence that the proposed method is capable of achieving the desired performance.

4.3 For measurements that require a calibrated system, the QAPP shall include specific calibration procedures, and the procedures for verifying both initial and continuing calibrations (including frequency and acceptance criteria, and corrective actions to be performed if acceptance criteria are not met).

5.0 QA/QC CHECKS

5.1 Data Quality Indicators

Statistical analyses shall be carried out on data obtained for all performance measurements. As part of the assessment of data quality, six data quality indicators (DQIs) can be used to interpret the degree of acceptability or utility of the data. At a minimum, the QAPP shall include a protocol for assessing the following DQIs, and acceptable limits and criteria for each of these indicators: representativeness, accuracy, precision, bias, comparability, and completeness.

The Testing Organization shall determine acceptable values or qualitative descriptors for all DQIs in advance of verification testing as part of the experimental design. The assessment of data quality will require specific field and laboratory procedures to determine the data quality indicators. All details of DQI selection and values shall be documented in the QAPP.

Representativeness

Representativeness refers to the degree to which the data accurately and precisely represent the conditions or characteristics of the parameter represented by the data. In this testing, representativeness will be ensured by executing consistent verification procedures. Representativeness will also be ensured by using each method at its optimum capability to provide results that represent the most accurate and precise measurement it is capable of achieving. For equipment operating data, representativeness entails collecting a sufficient quantity of data during operation to be able to detect a change in operations.

Accuracy

For water quality analyses, accuracy refers to the difference between a sample result and the reference or true value for the sample. Loss of accuracy can be caused by such processes as errors in standards preparation, equipment calibrations, loss of target analyte in the extraction process, interferences, and systematic or carryover contamination from one sample to the next. Loss of accuracy for microbial species can be caused by such factors as error in dilution or concentration of microbiological organisms, systematic or carryover contamination from one sample to the next, improper enumeration techniques, etc. The Testing Organization shall discuss the applicable ways of determining the accuracy of the chemical and microbiological sampling and analytical techniques in the Test Plan.

For equipment operating parameters, accuracy refers to the difference between the reported operating condition and the actual operating condition. For water flow, accuracy may be the difference between the reported flow indicated by a flow meter and the flow as actually

measured on the basis of known volumes of water and carefully defined times. Meters and gauges must be checked periodically for accuracy, and when proven dependable over time, the time interval between accuracy checks can be increased. In the Test Plan, the Testing Organization shall discuss the applicable ways of determining the accuracy of the operational conditions and procedures.

From an analytical perspective, accuracy represents the deviation of the analytical value from the known value. Since true values are never known in the field, accuracy measurements are made on the analysis of QC samples analyzed with field samples. QC samples for analysis shall be prepared with laboratory control samples, matrix spikes and spike duplicates. It is recommended for verification testing that the Test Plan include laboratory performance of one matrix spike for determination of sample recoveries. Recoveries for spiked samples are calculated in the following manner:

$$\% \text{ Recovery} = \frac{100(SSR - SR)}{SA} \tag{7-1}$$

where: SSR = spiked sample result
SR = sample result
SA = spike amount added

Recoveries for laboratory control samples are calculated as follows:

$$\% \text{ Recovery} = \frac{100(\,found concentration)}{true concentration} \tag{7-2}$$

For acceptable analytical accuracy under the verification testing program, the recoveries reported during analysis of the verification testing samples must be within control limits, where control limits are defined as the mean recovery plus or minus three times the standard deviation.

Precision

Precision refers to the degree of mutual agreement among individual measurements and provides an estimate of random error. Analytical precision is a measure of how far an individual measurement may be from the mean of replicate measurements. The standard deviation and the relative standard deviation recorded from sample analyses may be reported as a means to quantify sample precision. The percent relative standard deviation may be calculated in the following manner:

$$\% \text{ Relative Standard Deviation} = \frac{S(100)}{X_{average}} \tag{7-3}$$

where: S = standard deviation
$X_{average}$ = the arithmetic mean of the recovery values

Standard Deviation is calculated as follows:

$$\text{Standard Deviation} = \sqrt{\frac{(X_i - X)^2}{n-1}} \qquad (7\text{-}4)$$

where: Xi = the individual recovery values
X = the arithmetic mean of the recovery values
n = the number of determinations

For acceptable analytical precision under the verification testing program, the percent relative standard deviation must be less than ___ percent.

5.2 The QAPP shall list and define all other QC checks and/or procedures (e.g., detection limits determination, blanks, surrogates, controls, etc.) used for the project.

5.3 For each specified QC check or procedure, required frequencies, associated acceptance criteria, and corrective actions to be performed if acceptance criteria are not met shall be included in the QAPP.

6.0 DATA REPORTING, DATA REDUCTION, AND DATA VALIDATION

6.1 The reporting requirements (e.g., units) for each measurement and matrix shall be identified in the QAPP.

6.2 Data reduction procedures specific to the project shall be described, including calculations and equations.

6.3 The data validation procedures used to ensure the reporting of accurate project data to internal and external clients should be described.

6.4 The expected product document that will be prepared shall be specified.

7.0 ASSESSMENTS

7.1 Whenever applicable, the QAPP shall identify all audits (i.e., both technical system audits [TSAs] and performance evaluations [PEs]) to be performed, who will perform these audits, and who will receive the audit reports.

8.0 REFERENCES

8.1 References shall be provided in the QAPP in the body of the text as appropriate.